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THE INFLUENCE OF TEMPERATURE ON THE INFECTION AND DECAY OF SWEET POTATOES BY DIFFERENT SPECIES OF RHIZOPUS [1]

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INTRODUCTION

Temperature is regarded as an important factor in the infection and decay of sweet potatoes. Rather specific instructions regarding the proper temperatures at which sweet potatoes should be stored are given in the various publications dealing with storage. The temperatures recommended for curing and storing vary somewhat, but in general are as follows: For curing, 26° to 32° C.; for storing, 10° to 14°. Notwithstanding these recommendations, there is little information available regarding the relation of temperature to infection and decay by the various organisms responsible for the decay of sweet potatoes.

Among the most important organisms which cause this decay are some of the species of Rhizopus. The following species were found by Harter, Weimer, and Lauritzen (*3*)[2] to be capable of decaying sweet potatoes: *R. tritici* Saito, *R. nodosus* Namysl., *R. maydis* Bruderl., *R. oryzae* Went and Pr. Geerligs, *R. delemar* (Boid) Wehmer and Hanzawa, *R. arrhizus* Fischer, *R. reflexus* Bainier, *R. artocarpi* Racib., and *R. nigricans* Ehrnb.

Hanzawa (*1*) studied 14 species of Rhizopus, separating them into three thermal groups on the basis of such characters as the presence or absence of spore germination and the abundance of mycelial growth, and the presence or absence of fruiting on potato plugs at various temperatures and after different periods of time. He does not give the exact increments of growth with the rise in temperature or after different periods of time. No measurements are recorded. These three groups are as follows: (1) High-temperature group, *Rhizopus oryzae, R. arrhizus* Fischer, *R. chinensis* Saito, *R. japonicus* Vuillemin, *R. tonkinensi* Vuillemin, and *R. batatas* Nakazawa; (2) intermediate-temperature group, *R. nodosus, R. tritici, R. kasanensis* Hanzawa, *R. trubini* Hanzawa, and *R. usamii* Hanzawa; and (3) low-temperature group, *R. nigricans.*

Weimer and Harter (*7*) made an intensive study of the germination of spores in sweet potato decoction and of the mycelial growth and fructification on potato agar of 11 species of Rhizopus. They likewise separated the species studied into three thermal groups. Their grouping is slightly different from Hanzawa's and includes some species not studied by him and excludes others.

The work of these authors resulted in the following classification: (1) High-temperature group, *Rhizopus chinensis;* (2) intermediate-temperature group, *R. tritici, R. nodosus, R. delemar, R. oryzae, R. arrhizus,* and *R. maydis; and* (3) low-temperature group, *R. nigricans, R. microsporus* v. Teig., *R. reflexus,* and *R. artocarpi.*

Their results show the maximum, optimum, and minimum temperatures for spore germination, mycelial growth, and fructification, the increments of growth with the rise in temperature and increments of growth after different periods of time.

One difference between the two groupings is that Hanzawa places *Rhizopus oryzae* and *R. arrhizus* in the high-temperature group, while Weimer and Harter place them in the intermediate. This discrepancy is apparently not serious, for if Hanzawa's data are examined, one finds as good reason for placing these species in the intermediate group as with *R. chinensis;* in fact, a better reason if one eliminates *R. delemar,* which Hanzawa in his table still retains under *R. oryzae* and which shows a wider temperature range for sporulation than does *R. oryzae. R. chinensis* is sufficiently different from all the other species in its temperature response to place it in a group

[1] Received for publication June 30, 1924; issued June, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 810.

by itself, especially when it is considered in connection with the species Weimer and Harter (7) employed in their experiments.

The experiments recorded in the present paper had for their purpose (1) the determination of the optimum, maximum, and minimum temperatures for the infection and decay of sweet potatoes; (2) the measurement of the amount of decay with rise in temperature and after different periods of time; and (3) the determination of the time required for infection to take place, by six species of Rhizopus. These species are, *R. tritici*, *R. oryzae*, *R. maydis*, *R. reflexus*, *R. artocarpi*, and *R. nigricans* strains *a* and *b*.[3]

The reason for eliminating three of the species (*Rhizopus nodosus*, *R. delemar*, and *R. arrhizus*) found by Harter, Weimer, and Lauritzen (3) to be parasitic on the roots of sweet potatoes, and employing only the remaining six species, will now be considered. *R. tritici* and *R. nodosus* are almost identical in their temperature responses on culture media (7). The temperature responses of *R. oryzae* and *R. delemar* are likewise almost identical according to the data of Weimer and Harter (7) and there is scarcely any difference according to Hanzawa's data (1), excepting in fruiting, and here the difference is not great. Since these two pairs likewise behaved similarly in infection experiments (3), it was decided to eliminate one of each pair. *R. arrhizus* was not available at the time these experiments were conducted. *R. maydis* was selected because it is rather distinct in some of its characters, particularly in that it produces very few spores.

Rhizopus nigricans, *R. reflexus*, and *R. artocarpi* belong to the same thermal group, but show some variation in their temperature responses (6, 7). For example, there is a slight variation in the maximum, minimum, and optimum temperatures for spore germination, mycelial growth, and fructification of these three species (7). *R. artocarpi* is more successful in competition with *R. nigricans* at 14° C. than at 12°, and *R. reflexus* is more successful at 12° than at 14° (6).

Rhizopus nigricans is by far the most important organism of the group from the standpoint of the amount of decay it causes (6); in fact, in storage, it causes most of the soft rot decay of sweet potatoes.

MATERIALS

Single-spore strains of the above listed species were used in these experiments. The variety of sweet potatoes used was Little Stem Jersey. The potatoes were cured for 10 days at from 25° to 30° C. and stored at temperatures ranging mostly between 10° and 15°.

In these experiments it is important that the size and shape of the potatoes should be as nearly uniform as possible, otherwise any irregularities at the surface of the potatoes around the point of infection would alter the amount of decay. To be comparable in any two cases, the decay must be able to proceed within equal radii, which it can not do for any length of time if the potato is small and irregular in shape. It is not possible to obtain potatoes of identical size and shape, but it is possible to approach this condition sufficiently to make the results comparable. If one is comparing the amount of decay at several temperatures, there must be enough tissue available for the decay to proceed at the higher temperatures over sufficiently long periods to give opportunity for decay to take place at the lower temperatures.

APPARATUS

The apparatus described by Lauritzen and Harter (6) was used throughout these experiments.

Four types of experiments form the basis of this work, the methods employed varying with the type.

FIRST TYPE OF EXPERIMENT

This type of experiment was designed to determine the time required for infection, where infection depends on the organisms normally present on the potatoes. The potatoes in the condition in which they were received from the storage house, were wounded[4] to the same degree on the side of the roots at the point of the greatest diameter.

It is believed that wounding is the chief predisposing condition permitting infection by Rhizopus, and the methods employed in these experiments enable one to obtain some idea of the time required for infection to take place. They

[3] Strain as used here merely means that the organisms used were obtained from different sources and isolated from different hosts. Strain *a* was isolated from tomato by F. C. Meier, and strain *b* from sweet potato by one of the writers.

[4] The wounding was done with a special instrument with two blades, 1 cm. long and 0.2 cm. wide. The blades were mounted parallel to each other 1 mm. apart on a brass rectangular plate 1.5 by 1.5 cm., which served as a shoulder. On the opposite side of the plate was a handle used to shove the blades into the potatoes. The blades were pushed into the potatoes twice, as far as the shoulder permitted, the second time at right angles to the first.

at least permit of a comparison of the time required for infection at the various temperatures, because the wounding was the same at all temperatures and all the potatoes were grown and stored under identical conditions. These experiments were conducted the latter part of December and the first part of January so that there had been opportunity for some accumulation of spores on the potatoes, there being more spores (*6*) on the potatoes with the advance of the storage season.

the potatoes were wounded as in the first type of experiment and inoculated by dipping them in a spore suspension made from an equal number of cultures of *Rhizopus tritici* and *R. nigricans* (strain *a*).

Potatoes of uniform size and shape were selected, washed in tap water, dried in the laboratory for 24 hours, and divided into lots according to size and shape, each lot being weighed, wounded, and then inoculated by dipping them in a spore suspension.

TABLE I.—*Time required for the infection of sweet potatoes by Rhizopus tritici and R. nigricans at various temperatures*

Temperature (°C.)	Time required for infection	Number of potatoes inoculated	Number of potatoes infected	Remarks
32.5	43 hours	20	9	Size of lesions varied from 1 to 6 cm. in diameter. No infection in 24 hours.
27.5	do	20	7	Size of lesions varied from 0.5 by 1 to 4 by 5 cm. in diameter. No infection in 24 hours.
25.5	do	20	19	Size of lesions varied from 0.3 by 1 to 4 cm. in diameter. No infection in 24 hours.
23.5	do	20	14	Size of lesions varied from 1 to 3 cm. in diameter. No infection in 24 hours.
19.5	do	20	14	Infection just started. No infection in 24 hours.
18.0	do	20	6	Do.
15.0	3 days	20	12	Infection just evident. No infection evident after 2 days.
13.5	4 days	20	15	Lesions from 1 to 2 cm. in diameter.
9.0	7 days	20	20	Lesions varied from 3 to 10 cm. in diameter. No infection evident in 5 days.

Table I shows the number of days required for infection to take place at the various temperatures by *Rhizopus tritici* and *R. nigricans*. At temperatures of 18° C. and above infection took place in 43 hours and less. At 18° and 19.5° infection had just started in 43 hours. Above these temperatures infection started earlier, but in no case as early as 24 hours. At 15° three days were required for infection, and at 9° five to seven days. The time required for infection to take place at any of the temperatures is comparatively short. There will be some variation from this time, depending upon the degree of wounding, the time of season, and the conditions under which the potatoes are stored. When infection has once been established the time required for the potatoes to decay completely is comparatively short, four or five days at most. If potatoes are to be utilized after they have been badly wounded, they must be consumed within the first few days.

SECOND TYPE OF EXPERIMENT

These experiments were devised to measure quantitatively the amount of decay at various temperatures, where

After having been subjected to this treatment the potatoes were placed at the various temperatures and left for infection to take place and decay to develop. After the decay had developed to a suitable degree, the lots were weighed again, the decay removed by a spoon or some other blunt instrument, and the remaining undecayed portion weighed. The weight of the decay was obtained by subtracting the latter weight from the first weight. The reason for making the second weighing was to ascertain the loss or gain of water during the infection and incubation periods. There was a slight loss of water, especially at the higher temperatures, which was correlated to some extent with the amount of decay. There was a very slight gain of water at the lower temperatures in some cases where the humidity was exceptionally high. The humidity, however, was fairly comparable throughout the chambers, being slightly lower at the higher temperatures. The variation in loss of water was not sufficient to alter the general relations obtained.

It will be seen from the results recorded in Table II that the amount of decay increases rapidly with the rise in temperature, more rapidly than one

would expect from the van't Hoff law. The quantity of decay varies from nearly six times to many times as much at one temperature as at 10° below, except between 20.5° and 21.5° C. and at temperatures near the upper limit for infection.

TABLE II.—*Amount of decay at various temperatures by Rhizopus tritici and R. nigricans*

Temperature	Time after inoculation	Number of potatoes inoculated	Number of potatoes decayed	Weight of decayed tissue [a]
° *C.*	*Days*			*Grams*
37.0	4	50	48	3,511
32.5	4	50	46	5,906
29.0	4	50	42	4,600
26.5	4	50	29	2,626
21.5	4	50	37	925
20.5	4	50	40	885
14.5	7	50	34	1,878
11.5	7	50	43	254
6.5	12	50	50	680
4.0	12	50	50	109

[a] These weights were calculated on the basis of 30 potatoes from the decayed potatoes at each temperature.

There was less decay between 20.5° and 21.5° C., but these temperatures correspond to the lower limit of infection for *Rhizopus tritici* when this method of wounding and inoculation are used (*6*), and there is often a decrease of decay observed at these temperatures. It is not clear whether this is due to some factor associated with the pathogens or to resistance on the part of the host.

The increments of decay per degree are particularly high at temperatures between 11.5° and 14.5° C. and between 4° and 6.5°.

No isolations were made from these potatoes, but previous experience (*6*) would justify one in concluding that the organisms involved were *Rhizopus tritici*[5] and *R. nigricans*, *R. tritici* exclusively at 30° C. and above, and *R. nigricans* at 20° and below, the two overlapping between 20° and 30°.

THIRD TYPE OF EXPERIMENT

The procedure in these experiments was the same as in the second, except that the "well" method (*2*) of inoculation was employed. Forty-eight-hour old cultures of the organisms used, grown on 2½ c. c. of sweet potato decoction in test tubes at room temperature (20 to 25° C.), were introduced into "wells" 1 cm. in diameter and 4½ cm. deep. The "wells" were deep enough so the inoculum reached approximately the center of the potatoes. It was desired to start infection at or near the center so that the decay might proceed for some time before reaching the surface or skin of the potato as it proceeded outward from the point of infection. An indeterminable error results if the decay is permitted to reach the surface, because as soon as it reaches the skin at any point it stops in that direction, thus limiting the amount of decay.

From the data presented in Tables III and IV the six species studied can be separated into two groups according to their temperature responses: First, a high temperature, and second, a low temperature. *Rhizopus tritici*, *R. oryzae* and *R. maydis* belong to the former and *R. nigricans*, *R. reflexus* and *R. artocarpi* to the latter.

This relation is illustrated in Figures 1 and 2. Curves representing all of the organisms except *Rhizopus oryzae* are shown in Figure 1. *R. oryzae* and *R. tritici* are shown in Figure 2. The curves in Figure 1 were drawn from weighings (Table III) of decay at different temperatures after two days and those in Figure 2, from weighings made after three days. It will be seen from Figure 2 that *R. oryzae* belongs to the same group as *R. tritici*. The optimum temperatures for infection for the three species of the high temperature group lie between 32° and 35° C. (Table III and figs. 1 and 2), and those for the three species of the low temperature group between 18.5° and 23° C. (Table III and fig. 1).

In case of the high temperature group there is a close correspondence to the optimums obtained by Weimer and Harter (*7*) (*R. tritici*, 33° to 35° C., *R. oryzae*, 31° to 34°, and *R. maydis*, 30.5° to 32.5°, for mycelial growth on culture media. The optimum temperatures obtained by Weimer and Harter (*7*) for *R. nigricans* (23° to 26°), *R. reflexus* (26° to 28°), and *R. artocarpi* (26° to 28°) are somewhat higher than the optimum temperatures for infection obtained in the present experiments (*R. nigricans*, strains *a* and *b*, 18.5° to 23.5°, *R. reflexus*, 18.5° to 23°, and *R. artocarpi*, 23° to 24° (Table III). The data in connection with the last three species correspond to those recorded by Lauritzen (*5*) for *Puccinia graminis* Pers. var. *tritici* Erick. on wheat and *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. on beans, where the optimum temperatures for infection were slightly lower than optimum temperatures for germination of spores and growth of mycelium.

[5] *Rhizopus tritici* here is used in a collective sense and may include *R. nodosus*, *R. oryzae* and *R. delemar*. It is not possible from present knowledge to distinguish morphologically between any of these species

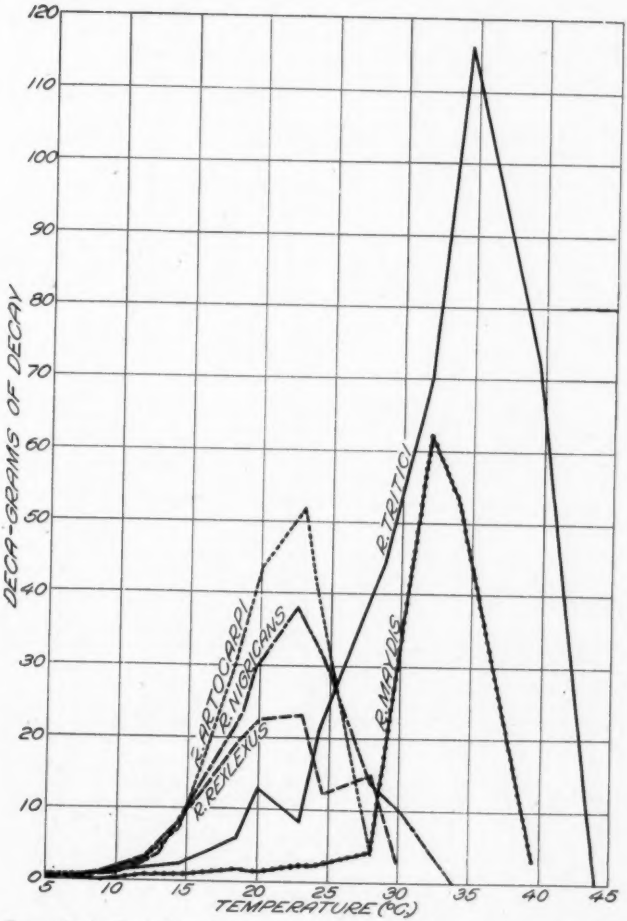

FIG. 1.—Curves showing the increments of decay by five species of Rhizopus with the rise in temperature after two days

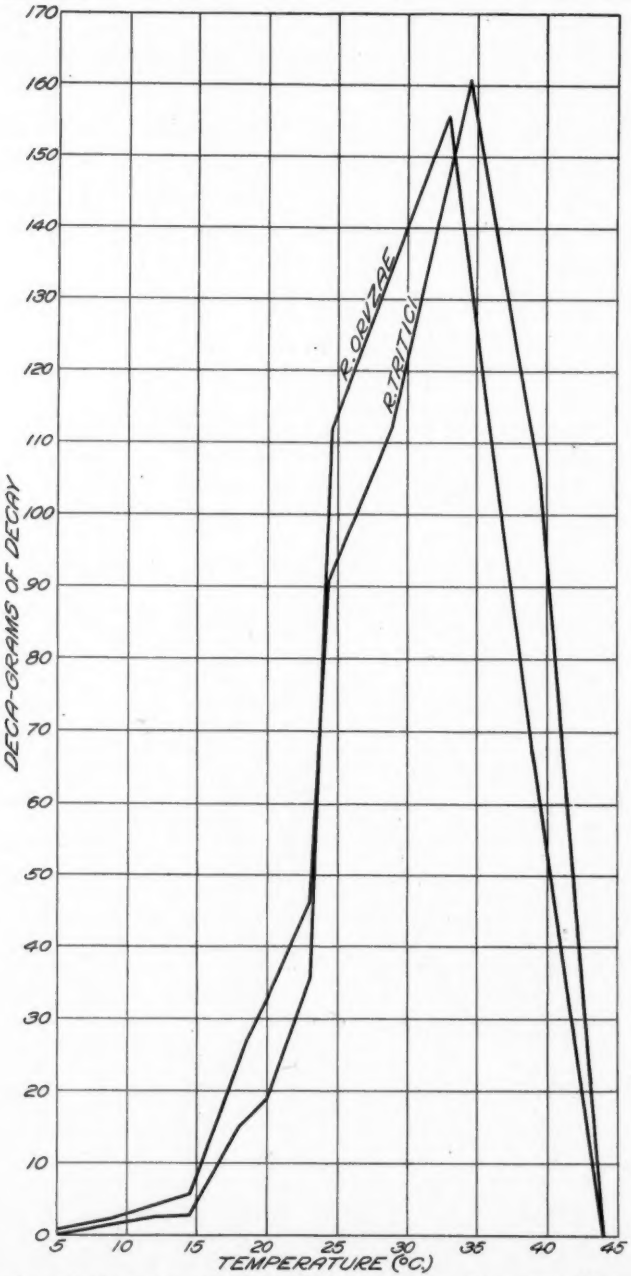

FIG. 2.—Curves showing the increments of decay by *Rhizopus tritici* and *Rhizopus oryzae* with the rise in temperature after three days

TABLE III.—*Amount of decay of sweet potatoes in grams produced by six species of Rhizopus at various temperatures and after different periods of time* [a]

Temperature	Rhizopus tritici					Rhizopus oryzae			Rhizopus maydis		Rhizopus nigricans strain *a*			Rhizopus reflexus		Rhizopus artocarpi	
	1 day	2 days	3 days	5 days	6 days	1 day	3 days	9 days	1 day	2 days	2 days	4 days	5 days	2 days	3 days	1 day	2 days
°C.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
39.5	155	729	1,051						22.5	31							
39.0						164.5	660										
34.5	212	1,162	1,607											6	0		
34.0									91	534							
33.0						228.8	1,555									0	
32.0	222	702	1,399						40.5	624						3.5	
29.8														28			
28.8	61	443	1,121								0	6	0				
28.0						122.2	1,295		21	45				149.1	100	43	40
24.5						77.7	1,119				36	245	526	122.5	191		
24.3	50	211	909														
24.0									17	23						135	408
23.0	23.5	83	462	1,196	888	72	356		18.5	25	36.1	578	770	231	273	85	516
20.0	32	127	329	1,176	698	24	188		11.5	12	79	362	264	224.1	348	68	432
18.5	39	62	272	780	890				13.5	16	72	508	635	194.5	650		
18.0						19.6	151									40	281.1
14.5		25	56	321	415	4	27.7		7	8				82.6	352	7	73
14.3											20	264	474				
12.0						0	25	275	11	8	15	242	321	33.5	231	4	32
9.0		12	24	68	72	0	16	20.0	7		7	27	59	12.1	56	0	10
5.0			8	14	10	0	0	5.5	0	7		0	0	0	12.5	0	9

[a] The data recorded in Table III are the average of two or more experiments of all the species (strain *a* of *Rhizopus nigricans*), except that of *K. maydis* where only one experiment was conducted, due to the difficulty of obtaining sufficient spores for a spore suspension from which to inoculate the tubes of sweet potato decoction. The weighings given represent the decay of four potatoes and in most instances they are the averages of two or more experiments. In some instances more than four potatoes were used, but the weighings given were calculated on the basis of four potatoes as a unit.

In general, it might be expected that the optimum temperature for infection would fall below the optimum for growth on culture media, because of the resistance offered by the hosts and the direct availability of the food in the culture media.

It has been noticed that at a temperature of 33° C. and above a larger percentage of unwounded sweet potatoes normally decay with Rhizopus soft rot than at lower temperatures. In other words, the resistance to Rhizopus is apparently broken down at the higher temperatures. This may explain why the optimum for decay by *R. tritici R. oryzae* and *R. maydis* is as high as for growth on artificial media.

Figures 3, 4, 5, 6, 7, and 8 show temperature curves of the six species indicated (*Rhizopus nigricans*, strain *a*) and represent the amount of decay after different periods of time. It will be noted from these curves (see also figs. 1 and 2) that the amount of decay does not increase in the same proportion as the rise in temperature in the early stages of decay in case of any of the organisms, there being a lag at the lower temperatures. There is a partial recovery of this lag with the lapse of time. The additional time required for the fungus to become established at the lower temperatures before decay actually begins may be a factor in producing the lag.

TABLE IV.—*Amount of decay of sweet potatoes caused by Rhizopus nigricans strain b, two days after inoculation* [a]

Temperature	Weight of decay	Number of potatoes inoculated	Number of potatoes decayed
°C	*Grams*		
38		20	
34		20	
30	103.2	37	12
28	138.0	47	28
24.5	312.0	47	40
22.5	379.6	47	39
19.7	296.0	47	42
18.8	234.4	46	43
13.0	38.4	47	47
11.8	24.0	47	47
10.0	9.2	47	46
7.0	5.2	46	46
5.0		47	

[a] In this table the results of two experiments are given and the weighings are calculated on the basis of four potatoes as in Table III. Controls were run in connection with these experiments. Sterile sweet potato decoction was introduced into the "well" instead of cultures of the organisms. The potatoes remained sound in all cases, indicating that the decay in case of the inoculated potatoes was due to the organism employed in the inoculum. Further proof of this indication is the fact that the decay of the respective organisms was always within their temperature growth limits on culture media (7).

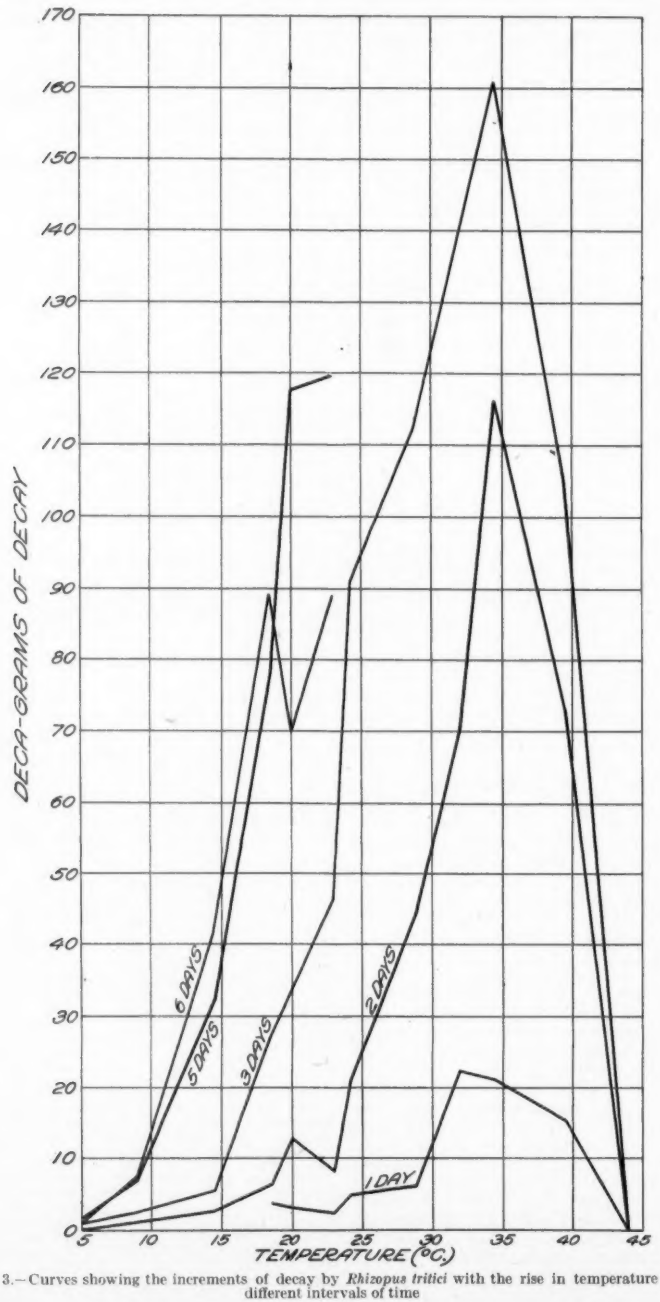

FIG. 3.—Curves showing the increments of decay by *Rhizopus tritici* with the rise in temperature after different intervals of time

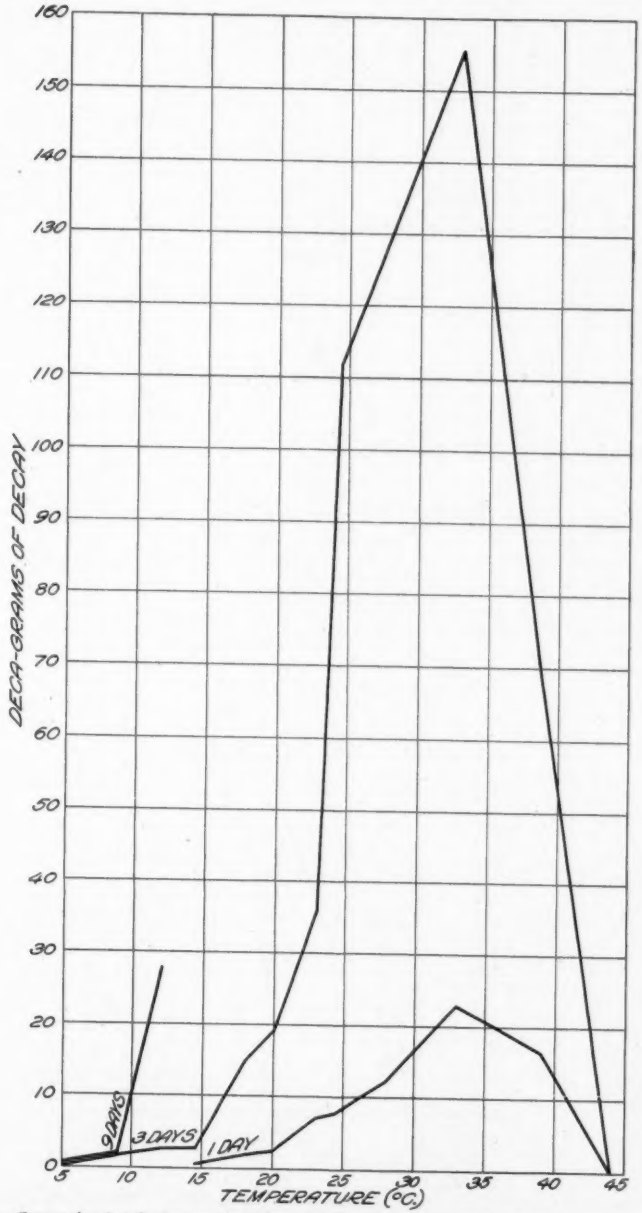

FIG. 4.--Curves showing the increments of decay by *Rhizopus oryzae* with the rise in temperature after different intervals of time

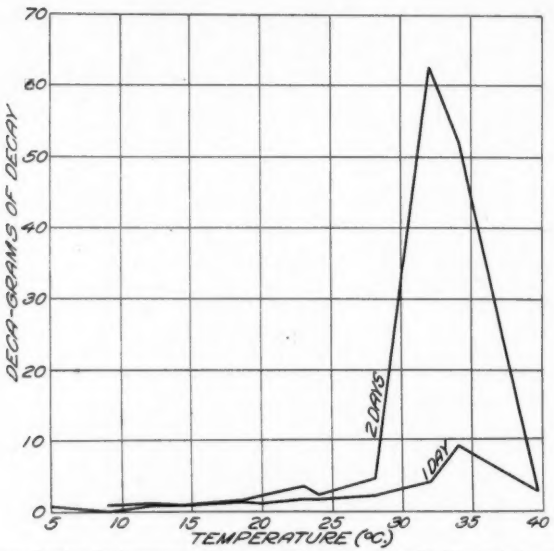

FIG. 5.—Curves showing the increments of decay by *Rhizopus maydis* with the rise in temperature after different intervals of time

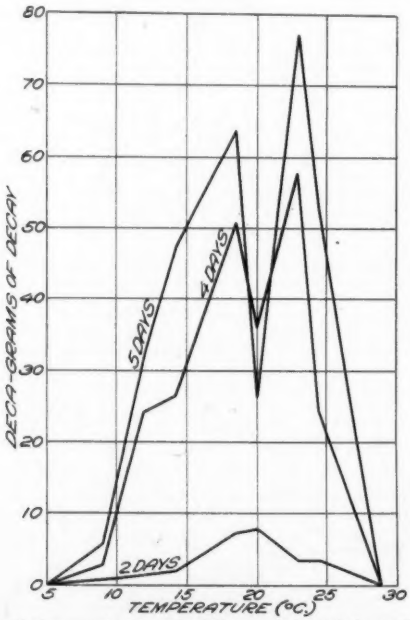

FIG. 6.—Curves showing the increments of decay by *Rhizopus nigricans* strain *a*, with the rise in temperature and after different intervals of time

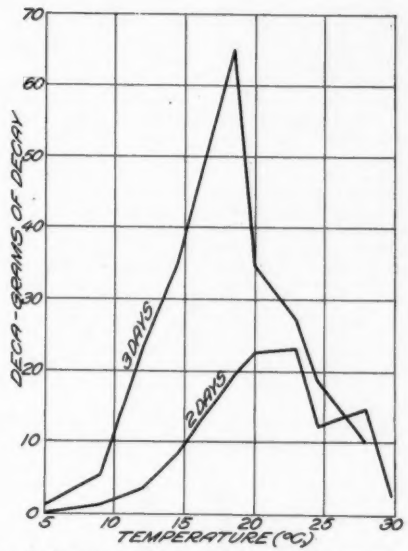

FIG. 7.—Curves showing the increments of decay by *Rhizopus reflexus* with the rise in temperature and after different intervals of time

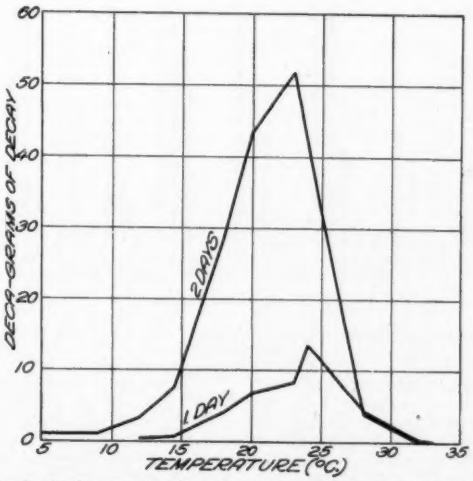

FIG. 8.—Curves showing the increments of decay by *Rhizopus artocarpi* with the rise in temperature and after different intervals of time

The amount of decay during the first day is much less than in the succeeding days, as would be expected. This may be accounted for (1) because some time is required for the fungus to become established on its new medium, and (2) because the operating surface increases rapidly with the lapse of time. Theoretically, because of the increasing operating surface, each succeeding interval of time should show an increased amount of decay. The data (Table III and fig. 9) in connection with *R. tritici* show this tendency, but not consistently. This inconsistency may be explained in part by the fact that in the later stages of decay there is a definite limit because of the size of the potatoes. would seem to indicate that the method employed here will give reasonably accurate results. It is true that some of the curves show some decided breaks, but the results taken as a whole are fairly consistent. It is not expected that they would be so uniform as those obtained in culture media, for here there are two organisms to be dealt with instead of one. Erratic behavior, so far as infection and the amount of decay in individual potatoes is concerned, is often observed. More uniform results probably would be obtained if a larger number of potatoes were employed, but the time element in setting up a particular experiment then becomes a source of error.

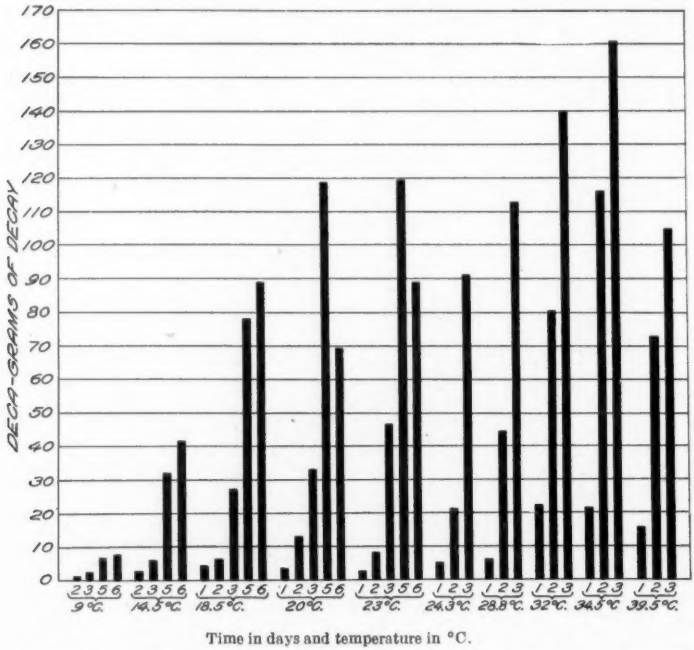

FIG. 9.—Graph showing the amount of decay by *Rhizopus tritici* at various temperatures and at different intervals of time

The extreme variation in the optimum temperatures given for the successive periods of time for any of the species is as follows: *Rhizopus reflexus*, 4.5° C. (Table III and fig. 7); *R. nigricans*, 3° (Tables III and IV and fig. 6); *R. tritici*, 2.5°, but is identical in two cases (Table III and fig. 3); *R. maydis*, 2° (Table III and fig. 5); *R. artocarpi*, 1° (Table III and fig. 8); and *R. oryzae*, 0° (Table III and fig. 4). This fact

The increase in the amount of decay with the rise in temperature is consistently high throughout these experiments as well as in the second type of experiment, higher as a rule than where other methods of measurements are employed, but it represents more nearly the actual loss involved than where the diameter of a spot is used as a measurement of the amount of decay; and even where the decay is a surface

phenomenon the loss due to the disease is more nearly represented by the area covered by the spot than by the diameter.

FOURTH TYPE OF EXPERIMENTS

These experiments were devised to determine the upper and lower temperature limits at which infection by the various organisms will take place, where the "well" method of inoculation was used.

UPPER TEMPERATURE LIMITS.—The upper temperature limits for infection by *Rhizopus tritici*, *R. oryzae*, and *R. maydis* are recorded in Table V, and for *R. nigricans*, *R. reflexus*, and *R. artocarpi* in Table III. *R. tritici* was isolated from each of eight potatoes at 42° C. but was not isolated at 44°. The upper limits found by Weimer and Harter (7) for mycelial growth were 42° to 45.5°.

Here is a case where the maximum temperature for infection exceeds that of mycelial growth on culture media, but it is entirely possible that the difference in the time element is sufficient to account for this apparent discrepancy especially since a vigorous culture (48 hours old) was used in the infection experiment as contrasted with a spore suspension drop in the mycelial growth experiment. It is possible, too, that the sweet potato any infection in the experiments in which it was used was 28.8° C. (Table III). Some infection took place by strain *b* at 30° after two days' time, but none at 34° (Table IV). This latter strain is the same as strain 4652, employed by Harter and Weimer in their physiological studies of strains of *Rhizopus nigricans* (4). They did not obtain growth on culture media of this strain at 30° in one day, but did obtain germination of spores at 31°.

TABLE V.—*Upper temperature limits for infection of sweet potatoes by Rhizopus tritici, R. oryzae and R. maydis*

Organism with which potatoes were inoculated	Time after inoculation	Number of potatoes inoculated	Number of potatoes decayed	Number of isolations made	Temperature	Organisms isolated	
						Number of times organism used in inoculation was isolated	Other organisms isolated
	Days				°*C.*		
Rhizopus tritici	2	8	8	7	44	0	Two plates were sterile and 5 gave bacteria.
Do	2	8	8	8	42	7	1 Rhizopus tritici plus bacteria.
Do	2	8	8	8	34	8	
Rhizopus oryzae	2	8	8	6	44	0	4 plates sterile, bacteria in 2.
Do	2	8	8	7	42	3	3 plates bacteria, 1 bacteria and Rhizopus.
Do	2	8	8	8	34	7	
Rhizopus maydis	2	6	6	6	44	0	Bacteria in all plates.
Do	2	8	8	8	42	1	Bacteria in 5 plates.
Do	2	8	8	8	34	6	Bacteria in 1, 1 sterile.

Rhizopus oryzae was isolated in 3 or 4 out of 8 potatoes at 42° C., but it was not isolated at 44°. The maximum found for mycelial growth by Weimer and Harter (7) was from 42° to 45.5°. *Rhizopus maydis* was isolated from 1 potato out of 6 at 42°, indicating that this temperature is near the maximum. It was not isolated at 44°. The maximum temperature for mycelial growth recorded by Weimer and Harter (7) was 40° to 44.5°.

The highest temperature at which *Rhizopus nigricans*, strain *a*, caused may form a better medium for growth than potato agar, the culture medium used by Harter and Weimer. It has been shown (7) that the maximum temperature may be affected by the culture medium.

Rhizopus reflexus caused some decay at 29.8° C. but none at 34.5° (Table III, and fig. 7). Weimer and Harter (7) found mycelial growth at 33°. It is possible that infection may have taken place at temperatures between 29.8° and 34.6°, but the drop in the amount of decay between 28.1°

and 29.8° was so marked as to indicate that the latter temperature is near the upper limit.

Rhizopus artocarpi caused slight, if any, infection at 32° C., considerable at 28.1°, but none at 33° (Table III and fig. 8); 32° therefore is apparently near the upper limit. It is possible that in case of the last three species the maximum might be raised some if a longer time were employed; but this seems improbable, however, for at these high temperatures if infection does not occur during the first two days the potatoes remain uninfected. Table VI contains a summary of cardinal temperatures

TABLE VI.—*Summary of the cardinal temperatures of the six species of Rhizopus studied*

Species	Minimum		Optimum	Maximum	
	Absent	Present		Absent	Present
	°C.	°C.	°C.	°C.	°C.
R. tritici		3.4	32 to 34.5	44	42
R. oryzae	5	9.0	33	44	42
R. maydis	5		32 to 34	44	42
R. nigricans (strain *a*)		3.4	18.5 to 23	28.8	24.5
R. nigricans (strain *b*)	5.0	7.0	22 to 23	34.0	30.0
R. reflexus		3.4	18.5 to 23	34.5	29.8
R. artocarpi	5	12.0	23 to 24	33.0	32.0

TABLE VII.—*Lower temperature limits for infection of sweet potatoes by Rhizopus tritici, R. oryzae, R. maydis, R. nigricans (strain a), R. reflexus and R. artocarpi*

Organism with which potatoes were inoculated	Time in days after inoculation	Temperature	Number of potatoes inoculated	Number of potatoes decayed	Number of isolations made	Organisms isolated	
						Number of times organism used in inoculation was isolated	Other organisms isolated
		°C.					
Rhizopus tritici	25	5.0	20	20	19	19	
Do	51	5.0	21	21	21	19	1 Botrytis, 1 Mucor.
Do	52	3.4	18	18	14	5	Botrytis and R. tritici mixed, 6; Botrytis, 1; bacterium, 2.
R. nigricans (strain *a*)	25	5.0	22	10	9	6	1 Rhizopus sp. and bacterium; 2 Mucor.
Do	51	5.0	20	20	20	20	
Do	52	3.4	18	18	9	3	6 Botrytis and Mucor mixed.
R. reflexus	25	5.0	21	21	16	12	4 Rhizopus sp. and bacteria. Rhizopus here probably reflexus.
Do	51	5.0	18	18	0	0	Decay too far advanced for isolation.
Do	52	3.4	18	18	18	18	
R. artocarpi	25	5.0	20	(a)	0	0	
Do	51	5.0	20	20	15	0	1 Mucor; 1 unknown organism; 10 Botrytis; 2 Penicillium; 1 bacterium.
R. maydis	51	5.0	14	14	12	0	7 Botrytis; 1 R. nigricans; 2 Mucor; 2 Mucor and Botrytis.
Do	52	3.4	18	18	12	0	8 Botrytis; 1 bacterium; 3 Mucor.
R. oryzae	51	5.0	18	17	15	0	9 Botrytis; 1 Mucor; 3 Penicillium. 2 unaccounted for.
Control	25	5.0	21	0	0		
Do	51	5.0	18	18	13		4 R. nigricans; 6 Botrytis; 3 Penicillium.
Do	52	3.4	18	13	13		2 R. nigricans; 7 Botrytis; 2 Mucor.

(a) Decay just started but dried up.

LOWER TEMPERATURE LIMITS.—The data relating to the lower temperature limits for all six species are recorded in Table VII. *R. tritici* will be seen to infect sweet potatoes readily at 5° C. by this method of inoculation, it being obtained 38 times out of 40 isolations. At 3.4° it was only obtained 5 times out of 14 isolations. This temperature is probably very near the lower limit for infection by this organism because, although Weimer and Harter (*7*) found that *R. tritici* spores germinated at 1.5°, no growth of mycelium in culture medium took place at this temperature, but mycelial growth did take place at 6.5°. No observations were made on mycelial growth between these temperatures.

There was no infection by *Rhizopus oryzae* after 51 days at 5° C. In Table III there is some evidence of infection at 5°, but the loss in weight (5.5 gm.) recorded after nine days may have been due to maceration, brought about by the enzyme that may have been present in the cultures used for inoculation and a slight amount of sweet potato decoction that was remaining in the "well." [6] Some decay (Table III) did take place at 9°. One is therefore safe in assigning some temperature between 5° and 9° as the lower temperature limit for infection. Weimer and Harter (*7*) found germination of spores at 9° but none at 7° and growth of mycelium at 11° but none at 7.5°.

It will be observed that the lower temperature limit for infection by *Rhizopus oryzae* is somewhat higher than that for *R. tritici* (Table VII). These results correspond to those obtained by Hanzawa and Weimer and Harter for spore germination, mycelial growth, and fructification (*1*), (*7*).

Rhizopus maydis caused no infection at 3.4° C. or at 5° after 51 and 52 days, respectively. Although the lower temperature limit has not been accurately determined in this case, it can be definitely said that no infection took place at and below 5°. According to the data recorded in Table III there is a wide range over which scarcely no infection takes place. What would have happened if more time (two days) had been employed can not be said. In any case the behavior exhibited is unusual. Even at temperatures from 18.5° to and including 28° scarcely any infection had taken place after two days. Weimer and Harter (*7*) obtained growth of mycelium at 7.4 but not at 1.5°.

Rhizopus nigricans strain *a* infected 26 or 27 potatoes out of 29 at 5° C. Nine of these potatoes were held 25 days and 20 for 51 days at this temperature. It infected only 3 potatoes out of 9 at 3.4° after 52 days, indicating that this temperature is near the lower temperature limit, because at the higher temperatures it was not difficult to obtain 100 per cent infection. These results are confirmed by earlier results obtained by the writers (*6*) with this strain where very slight infection was obtained at 3.5° but none below. Weimer and Harter (*7*) obtained germination of *R. nigricans* spores at 1.5° and mycelial growth at 6.5°. They give no records of mycelial growth between these temperatures.

Rhizopus reflexus infected 12 out of 16 potatoes at 5° C. Rhizopus was isolated from the other four potatoes, but being contaminated with bacteria their identity was not certain, but it seems probable that they were *R. reflexus*. Another lot showed 100 per cent infection, but the decay at the time of observation was too advanced for isolation work. At 3.4° *R. reflexus* was isolated from every potato out of 18 held at this temperature. It will thus be seen that the lower limit for infection by *R. reflexus* is probably lower than that of *R. nigricans*. Weimer and Harter (*7*) found that not only would the spores of *R. reflexus* germinate at 1.5° but the mycelium would likewise grow at this temperature.

Rhizopus artocarpi caused no infection at 5° C. Whether or not the fungus had actually established host relations at 5° and 9° in the experiments recorded in Table III can not be said definitely, because no isolations were made. Twelve degrees is the lowest temperature at which infection has been recorded, but it seems probable that it can be obtained at a lower temperature.

DISCUSSION AND CONCLUSIONS

Knowledge of the time required for infection to take place is often an important factor in the loss due to diseases. Such knowledge is especially valuable in connection with diseases of fruits and vegetables in storage and

[6] This statement likewise applies to some of the weighings recorded in Tables III and IV in connection with the other species at the lower temperatures. Where the weighings are 8 gm. and less the loss of weight can be explained on this basis. At the upper limiting temperatures the decoction dries up sooner and the action of the enzymes is less evident unless infection actually takes place.

transit, where environmental conditions are or can be under partial control. It may serve as a means of preventing infection, reducing the amount of decay, and affording a basis for predicting the amount of loss that might be expected to take place under certain conditions. Sweet potatoes exposed to cold storage conditions at temperatures between 0°C. and 5° for a period of two to six weeks will invariably become infected with *Mucor racemosus* Fes. (*6*). They can be allowed, however, to remain at these temperatures for a period of 10 days if they are removed to temperatures ranging from 10° to 15° without becoming infected with this organism.

Where sweet potatoes show some infection with black rot, it can be predicted with certainty that if they are exposed to temperatures of 18° to 30° C. the loss will be greatly aggravated. If these potatoes are held at temperatures ranging from 10° to 12° the advance of the decay will be greatly checked.

The time required for infection with Rhizopus is not only short, but there is another discouraging feature that makes it difficult and generally impossible to prevent infection where the conditions are favorable to infection. Although sweet potatoes generally must be freshly wounded to permit of infection by Rhizopus, it is only under special conditions, namely, that the wounding be shallow and clear cut and that the humidity be high, 96 to 100 per cent, that infection can be prevented after potatoes have been wounded.

It is possible to retard infection five or six days after the potatoes have been wounded (Table I) by placing them at 9° C. Losses can thus be prevented if the potatoes are consumed within this time. This period may be extended where the potatoes are not as severely wounded as they were in these experiments, which probably is usually the case. The period might also be extended if few spores were present or shortened if the spores were abundant. After infection takes place there is little hope of saving the potatoes unless they are used immediately, because the decay progresses very rapidly even at the lower temperatures.

In Table VI is given a summary of the optimum, minimum, and maximum temperatures for the six species of Rhizopus studied.

The optima for *R. tritici*, *R. oryzae*, and *R. maydis* are almost identical with those obtained by Weimer and Harter (*7*) for mycelial growth, while the optima for *R. nigricans*, *R. reflexus*, and *R. artocarpi* fell from 3° to 5° below those for mycelial growth.

This relation of the optima for infection to the optima for mycelial growth seems to be correlated with the resistance of the host which apparently breaks down at the higher temperatures.

There is a close correspondence between the temperature limits for infection and those for mycelial growth on culture media recorded by Weimer and Harter (*7*), particularly the upper limits. In some instances (*R. maydis*, *R. nigricans*, strain *b*, at high temperature and *R. tritici*, *R. oryzae* and *R. nigricans* at low temperatures) infection was obtained at temperatures beyond the limits where mycelial growth is recorded; but the next higher or lower temperatures employed in their experiments amounted to several degrees, (except in the case of *R. nigricans*, strain *b*,) and it is quite possible that had they employed intervening temperatures mycelial growth may have been obtained. Only in the case of *R. nigricans*, strain *b*, was infection obtained as high or low as the temperature recorded for the absence of mycelial growth. *R. nigricans*, strain *b*, caused infection at 30° C. in two days but did not exhibit mycelial growth at this temperature in one day. Spore germination was obtained at 31°, so that difference in time in the two instances may account for these results. *R. maydis* is the exception that does not show a close correlation between the temperature limits for infection and those for mycelial growth. It shows a wide range at the lower temperatures over which it causes no infection. The explanation of this behavior is not apparent.

The maximum temperature recorded here for infection for *Rhizopus tritici* is not quite so high as that given by Lauritzen and Harter (*6*), the former being 42° C. and the latter 44° C. It will be noted however in the latter case that *R. tritici* was isolated only three times out of 34 isolations. In any case the cardinal temperatures given are not to be regarded as definite as the figures may indicate. One must take into consideration the variations of the pathogen, host, and the experimental conditions in connection with these experiments, as well as with any physiological experiments.

The temperature range for infection by any of the low-temperature species combined with any of the high-temperature species is wide, as was

the case with *Rhizopus tritici* and *R. nigricans* (Type I) (*6*), covering temperatures varying from 3.4° to 12° C. at the lower limits to 42° at the upper. *R. tritici* has the widest range of any of the species; in fact, is as wide as the combination of any two of the low and high temperature species. It is quite possible that *R. reflexus* may be able to infect at a lower temperature than *R. tritici*, but these data do not show that it can do so.

The maximum and optimum temperatures of *Rhizopus tritici*, *R. oryzae*, and *R. maydis* are several degrees higher than those of *R. nigricans*, *R. reflexus*, and *R. artocarpi*. These six species can thus be separated into a high and low temperature group. The former group corresponds to the intermediate-temperature group of Weimer and Harter (*7*) and the latter to their low-temperature group.

The two strains of *Rhizopus nigricans* show a slightly different response to temperature. Strain *b* has a somewhat higher maximum after a given time than strain *a*. The optimum of strain *b* falls within the optimum range of strain *a*, the latter having a slightly wider range. The lower temperature limit of strain *b* was not determined except for the period of two days, which was too short to be accurate. Considerable time is required for infection at temperatures below 5° C.

The increments of decay with the rise in temperature are too large to claim any relation with the van't Hoff law, at least directly. In some instances the amount of decay is 10 times as great at one temperature as at 10° below.

Notwithstanding the fact that a comparison frequently is made between the rate of chemical reaction and the rate of growth, there seems upon careful analysis of the phenomena involved in two cases to be no real justification for such a comparison. In the first case pure chemical reactions are under consideration, while in the second numerous reactions, not all chemical, too complex for perfect analysis with present knowledge are involved.

In the present experiments the surface exposed to the action of the fungus is never the same at the various temperatures at any moment after the decay begins. The decay radiates outward in all directions from the point of infection where the "well" method of inoculation is employed, and in all directions where there is any tissue to decay where infection begins on the side of the potato forming a spherical mass of decayed tissue. The area of attack corresponds to the surface of this sphere. Not only does the formation of this sphere at the higher temperatures commence earlier than at lower temperatures but it enlarges more rapidly because of the greater rate of decay due to the effects of temperature, so that the discrepancy in the area exposed to the action of the fungus at the various temperatures becomes larger and larger with time.

Where the decay is a surface phenomenon the area exposed to the action of the fungus likewise increases with the lapse of time. In this instance the area exposed conforms more to the area of a circle instead of that of a sphere.

There is no reason to expect any correlation between linear measurements of growth and chemical reactions. In the latter case one has to do with mass; in the former, with distance. The mass in case of any decay will vary with the host and the character of the disease, in other words, where two spots are of the same dimensions in two different tissues, the mass may be totally different.

Other factors, such as the amount of enzymes secreted at the various temperatures, the passing of the hyphae of the fungus out of the area of the end products, the nature of the decay, and the character of the host and the fungus, play a part in these reactions. These factors are absent in pure chemical reactions.

SUMMARY

The data regarding the influence of temperature upon infection and decay of six species of Rhizopus are presented in this paper.

The time required for Rhizopus to infect sweet potatoes wounded but not artificially inoculated varies from five to seven days at 9° C. to 43 hours and less at 18° to 32°. This time would be expected to vary with the amount of wounding and the number of spores present on the potatoes.

The six species of Rhizopus studied can be placed into two groups according to their temperature responses, as follows: A high-temperature group, *R. tritici*, *R. oryzae*, *R. maydis*, and a low-

temperature group, *R. nigricans*, *R. reflexus*, and *R. arlocarpi*. The optima for the high temperature group vary from 32° to 35° C., the maxima are about 42° and the minima vary from 3.4° to 9°; the minimum for *R. maydis* has not been definitely determined. The optima for the low temperature groups vary from 18.5° to 24°, the maxima from 30° to 34.5°, and the minima from 3.4° to 12°. The extreme temperature limits over which the entire group will infect sweet potatoes are between 3.4° and 42°. *R. tritici* will infect over this entire range. The two strains of *R. nigricans* have a slight difference in their temperature response, the one having a slightly higher maximum than the other. The increase in the amount of decay with the rise in temperature is large, being four to ten times as much as one temperature as at 10° below.

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TIME OF YEAR TO PLANT MOTHER BEETS FOR SEED PRODUCTION[1]

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INTRODUCTION

In the development of the sugar-beet seed industry for this country, the quantity and quality of seed should be among the first considerations. Some American seed companies have failed or lost heavily because of insufficient yield or poor quality of seed, which may have been due to many causes, such as poor storage, unfavorable weather conditions, poor care, or late planting. At the Salt Lake City Station (Utah) the writer has found that the quantity and quality of seed produced may vary greatly with the time of the year when the mother beets are planted. Beets planted early produce abundant seed, while those planted later produce little or no seed.

MATERIAL

Beets of the same variety and uniform as to weight, per cent of sugar, shape, and physical conditions were selected, analyzed, stored over winter, and planted during the next summer. The beets planted as late as September were apparently in as good condition as those planted in March. The apparently perfect condition of all beets at the time of planting was indicated by the fact that an unusually high percentage of the beets grew, all living beets produced seed, and there were no "trotzers," this being the reverse of conditions experienced by some investigators who have had from 50 to 90 per cent vegetative and unfruitful beets.

EXPERIMENTS

During 1922 and 1923 similar lots of beets were taken from storage each month, from March to September, inclusively, and carefully planted in rows on the same plot of ground. The soil was kept at a uniform moisture content throughout the summer. Records were kept on the physical condition and development of the plants. Data for the following physical conditions were obtained: Soil and air temperatures, soil moisture, evaporation, relative humidity, wind movement, sunlight conditions, and relative length of day. The plant was studied as to storage material, root, vegetative leaf, seed stalks, flowering, and quantity and quality of seed produced.

TABLE I.—*Average data for vegetative growth and seed production for 1922–1923*

Time of planting	Vegetative foliage	Height of seed stalks	Number of seed stalks per beets	Weight of seed produced per beets	Germination of balls
	Per cent	*Cm.*		*Gm.*	*Per cent*
Mar. 1	100	170	18	290.0	97.0
Apr. 1	90	170	18	293.0	99.0
May 1	80	125	17	228.2	96.0
June 1	40	90	12	81.0	71.0
July 1	10	80	7	40.0	50.0
Aug. 1	0	40	3	(a)	--------
Sept. 1	100	40	3	(a)	--------

a These did not mature.

The results given in Table I indicate that the quantity and quality of seed depend on the time of year when the beets are planted. Beets planted early produced the most and best seed. There appears to be very little difference in the results obtained from April and March plantings in these respects. The data also indicate that the quantity and quality of seed produced is directly proportional to the vegetative foliage (except for very late planting), height of seed stalks, and number of seed stalks developed during the second year. Shaw's [2] work indicates that the vegetative development is indirectly proportional to the

[1] Received for publication July 2, 1924; issued June, 1925.

[2] SHAW, H. B. CLIMATIC CONTROL OF THE MORPHOLOGY AND PHYSIOLOGY OF BEETS. Sugar 19: 387–391, 431–434, illus., 1917; 20: 23–27, 68–70, 109–112, 150–154, illus., 1918. 1917–18.

seed produced; but it appears that this is true only when dealing with beets which tend to be vegetative or are "trotzers."

The increase in production of seed in proportion to the increase in number of seed stalks per beet agrees well with the results of other investigators.[3] The increase in the number of seed stalks per beet is probably due to the inhibition of the terminal bud during the early growth period and the mobilization of an excess of available food material which gives the outer crown-buds time and favorable opportunity for development, thereby giving rise to an increased number of seed stalks. Further evidence of this is given by the fact that single or few seed stalks can be developed on beets at will. As a result there occurs the single or few seed stalk habit of annual or bolting beets, mother beets which are planted in the heat of summer, mother beets with outer crown buds destroyed due to excessive drying, and also annual or mother beets which are subjected to forced reproduction in the greenhouse at high temperatures. The factors which lead to the single or few seed stalk habit of beets are those which tend to immediate reproduction, thus forcing the terminal and retarding the outer crown buds.

Plate 1 shows that the seed spikes, seed balls, and floral bracts vary considerably with the period of year the beets are planted. Beets planted early tend to produce compact seed spikes and large seed balls, with small bracts or none. Beets planted later in the season tend to produce elongated floral stalks, smaller seed balls, and an increased number of larger seed bracts. The seed bracts on the beets planted in July and August tend toward vegetative leaves, and are much enlarged. If beets planted as late as September produce seed stalks, they are of a vegetative rosette type, as illustrated in Plate 1, E. These data indicate that later planting results in a tendency for beets to be more vegetative even when producing seed.

The rate of development of seed stalks and seed is given in Table II. The reproductive development was greatly retarded in the beets that were planted early, as indicated by the days elapsing from time of planting to time of flowering. Beets planted in the heat of summer tended to produce seed within a short period, while beets planted in the fall were very irregular in seed production and did not tend to develop reproductive organs. Photoperiodism experiments were arranged in the hope of finding some explanation of this increased seed production resulting from early planting. Both individual beets and lots of beets were exposed to the various lengths of days during spring, summer, and fall. An increased length of days during the spring had no noticeable effect upon the beets up until June 1, two months after planting. It was evident that there were other more important factors limiting the growth of reproductive organs during this period. From June 1 on, the seed stalks of beets subjected to light were probably more of a seed type, and flowered about one week earlier than beets not subjected to light. Beets planted late in the season and exposed to increased illuminations made more rapid seed-stalk growth and tended toward more reproductive seed stalks with greater per cent of late seeds. This is well illustrated by an individual beet (pl. 2, A) planted in August, the right side of which was exposed to six hours extra illumination from Sept. 8 to Oct. 15. It thus appears that light is an important factor in the lengthening of seed stalks when other limiting factors are not present.

TABLE II.—*Development of seed stalks and seed, 1922–23*

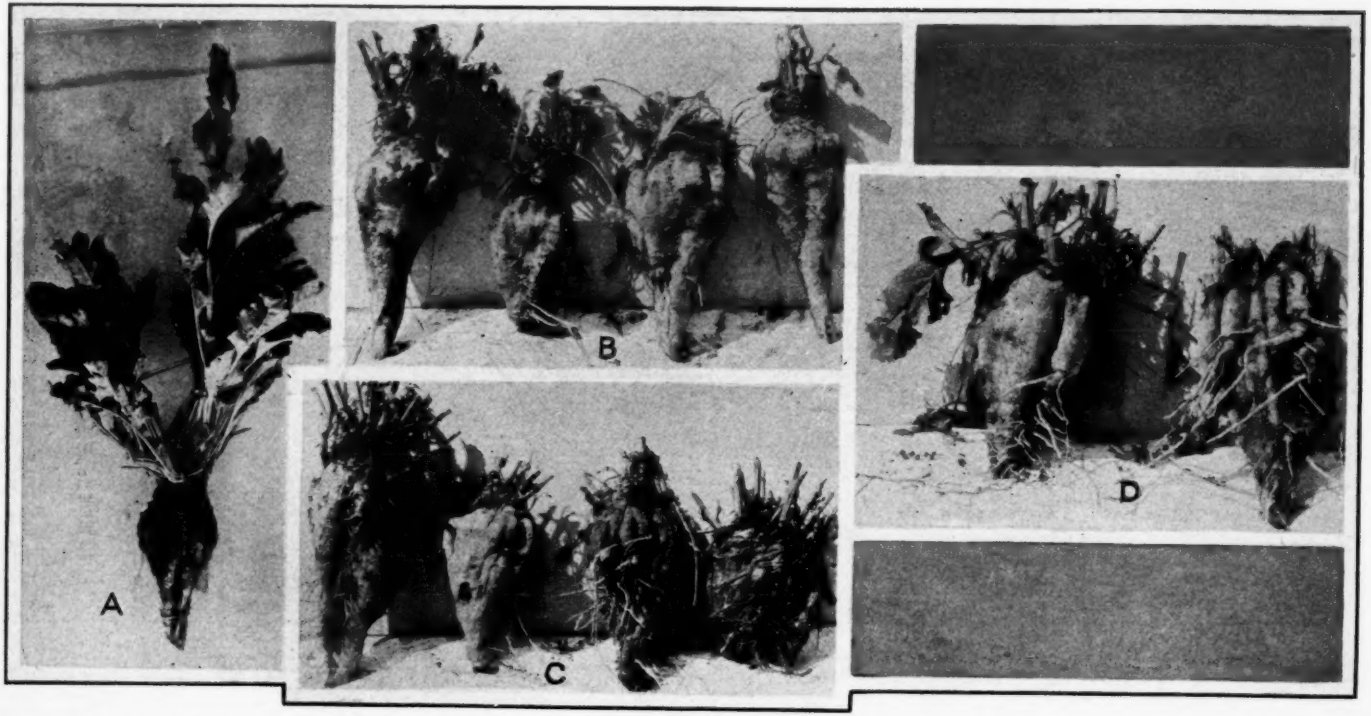
Time of planting	Time required to reach final height	Time after planting beet to date of first flowering	Length of flowering period
	Days	*Days*	*Days*
Mar. 1	120	113	17
Apr. 1	90	82	19
May 1	60	52	24
June 1	45	42	25
July 1	30	30 to 60	20 to 75
Aug. 1	30	Irregular	Indefinite
Sept. 1			

The meteorological data as to wind movement, relative humidity, and evaporation, gave no further explanation than did the effect of increased illumination. While these are all of importance, there seems to be other more important factors which require consideration. It was not until the beet roots were removed at different periods during the season that it was learned that those beets which produced the best quantity and quality of seed had previously produced the most marked root development.

Plates 2 and 3 show the root development of beets planted from April to

[3] HARRIS, F. S., and HOGENSON, J. C. SOME CORRELATIONS IN SUGAR BEETS. Genetics 1: 334–347. 1916.

A. Seed spikes from beets planted in March and April.
B. Seed spikes from beets planted in May.
C. Seed spikes from beets planted in June.
D. Seed spikes from beets planted in July and August.
E. Seed spikes from beets planted in September.



A.—Effect of additional illumination on seed stalk development B.—Beets planted June 1 C.—Beets planted May 1 D.—Beets planted April 1

September. The root development of beets planted March 1 was similar to that of beets planted April 1. Beets planted in April and May showed some enlargement of the old root and especially a marked development of new side roots. These side roots on the beets planted early are many and robust, while they are few, very fine, and threadlike on the beets planted later in the season. In this connection it was learned from the soil and air thermograph records that the enlarged root systems were developed at a time when the soil and air temperatures were low, and that the meager threadlike root systems were developed when the soil temperature and air temperature were high. The mean air and soil temperatures for the year 1923 are given in Table III. Plate 3, C, shows extensive root development of beets planted in September, at a time when soil temperature corresponded to the soil temperature of May. It is probable that these beets would produce an abundance of seed if September and October were followed by months of high temperatures, such as June and July.

TABLE III.—*Mean air and soil (6-inch depth) temperatures for 1923*

Month	Mean air	Mean soil
	°C.	°C.
Mar.	3.9	3.0
Apr.	9.8	9.0
May	16.7	14.8
June	18.2	16.2
July	24.5	21.5
Aug.	22.6	20.7
Sept.	18.1	15.6
Oct.	11.6	9.7

In order to verify this effect of temperature more carefully, beets were planted under controlled moisture and temperature conditions ranging from 15° to 30° C. The result was that the more marked side-root development occurred between 15° and 30°. The side roots developed at higher temperatures were thin and threadlike, as shown in Plates 2, B, and 3 A, B.

In order to verify more carefully the effect of illumination on side-root development, and indirectly on seed development, beets were planted under controlled moisture and temperature conditions favorable for side-root development, and the quantity of illumination varied. Beets exposed to short or long illumination periods produced more robust and more numerous side roots than beets exposed to darkness (pl. 4, A and B). The beets planted April 1, in the field and exposed to 24-hour illumination until June 1, showed no greater root development than a similarly planted lot exposed to only 12-hour illumination. It appears that under favorable temperature conditions the usual day illumination of April and May is quite sufficient for maximum side-root development, and that the amount of illumination may not be as important as the presence or absence of illumination.

It was learned that an abundant root-bud and side-root development could be forced under controlled temperature and moisture conditions by removing crown buds (pl. 4, C). It was also possible to force crown development by removing root-bud tissues. It was further noted that temperatures of 15° and 20°C. inhibited seed-stalk development, while these same temperatures favored the development of side roots and vegetative leaves. Likewise, temperatures of 25° C. retarded the development of side roots and vegetative leaves, due to the fact that it forced seed-stalk development. Even though vegetative organs, such as leaves and side roots, may lengthen faster at higher temperatures, these same organs develop best at lower temperatures.

DISCUSSION

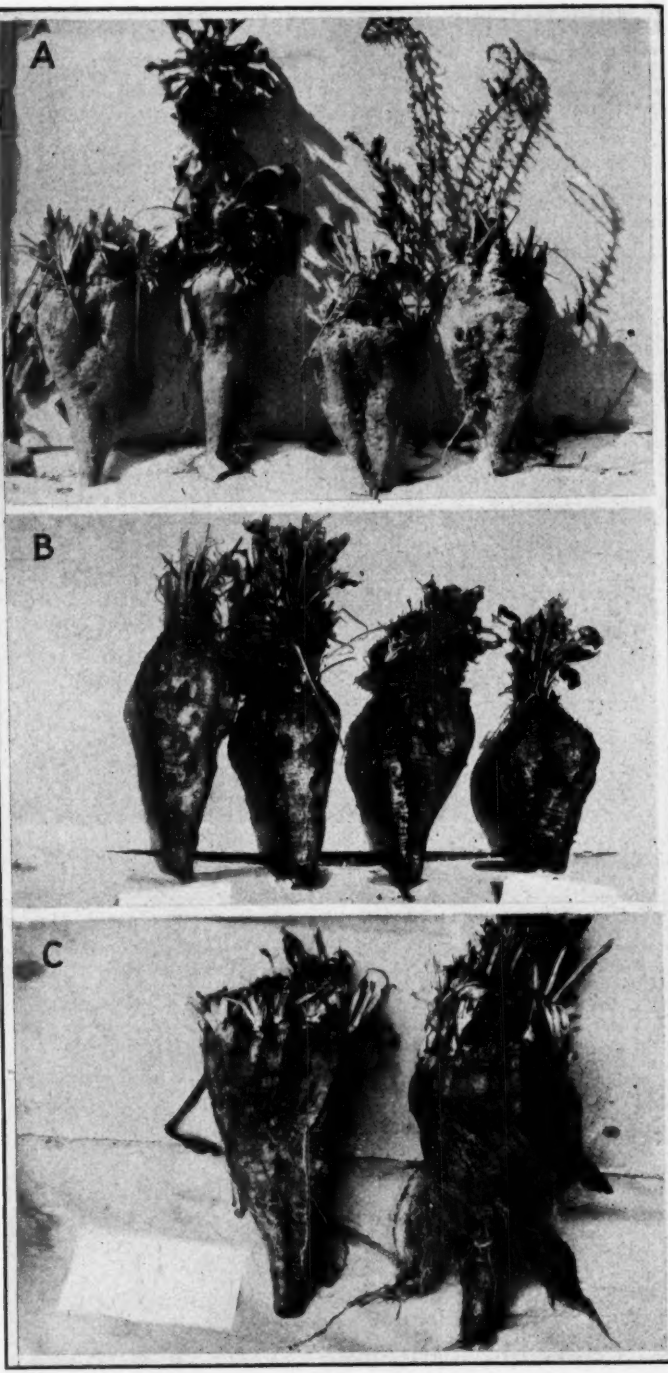
In explanation of this increased seed production resulting from early planting, Briem [4] states that the growth period of the beet from planting to blossoming time should be twice as long as the period from blossoming to ripening of seed, and that this period must have a slow, steady rise of temperature. Shaw [5] concluded from his experiments that the beets must pass through a period of restrained growth, at a temperature between 2.75° and 10° C., in order for the flower buds to develop. That this is not necessary is shown by the fact that beets stored at 1.7° or above 15° C., and planted under high temperatures in June, July, and August were not "trotzers" and produced seed.

The true explanation of the relation of increased seed production with early planting of beets will probably be found in many influencing factors. It seems that certain fundamental factors, such as moisture, temperature, and aeration, must be supplied before the

[4] BRIEM, H. ZEIT DES AUSPFLANZENS UND DER SAMENERTRAG BEI MUTTERRÜBEN. Österr. Ungar. Ztschr. Zuckerindus. u. Landw. 27: 685–690. 1898.

[5] SHAW, H. B. CLIMATIC CONTROL OF THE MORPHOLOGY AND PHYSIOLOGY OF BEETS. Sugar 19: 387–391, 431–434, illus., 1917; 20: 23–27, 68–70, 109–112, 150–154, illus., 1918. 1917–18.

A.—Effect of additional illumination on seed stalk development B.—Beets planted June 1 C.—Beets planted May 1 D.—Beets planted April 1

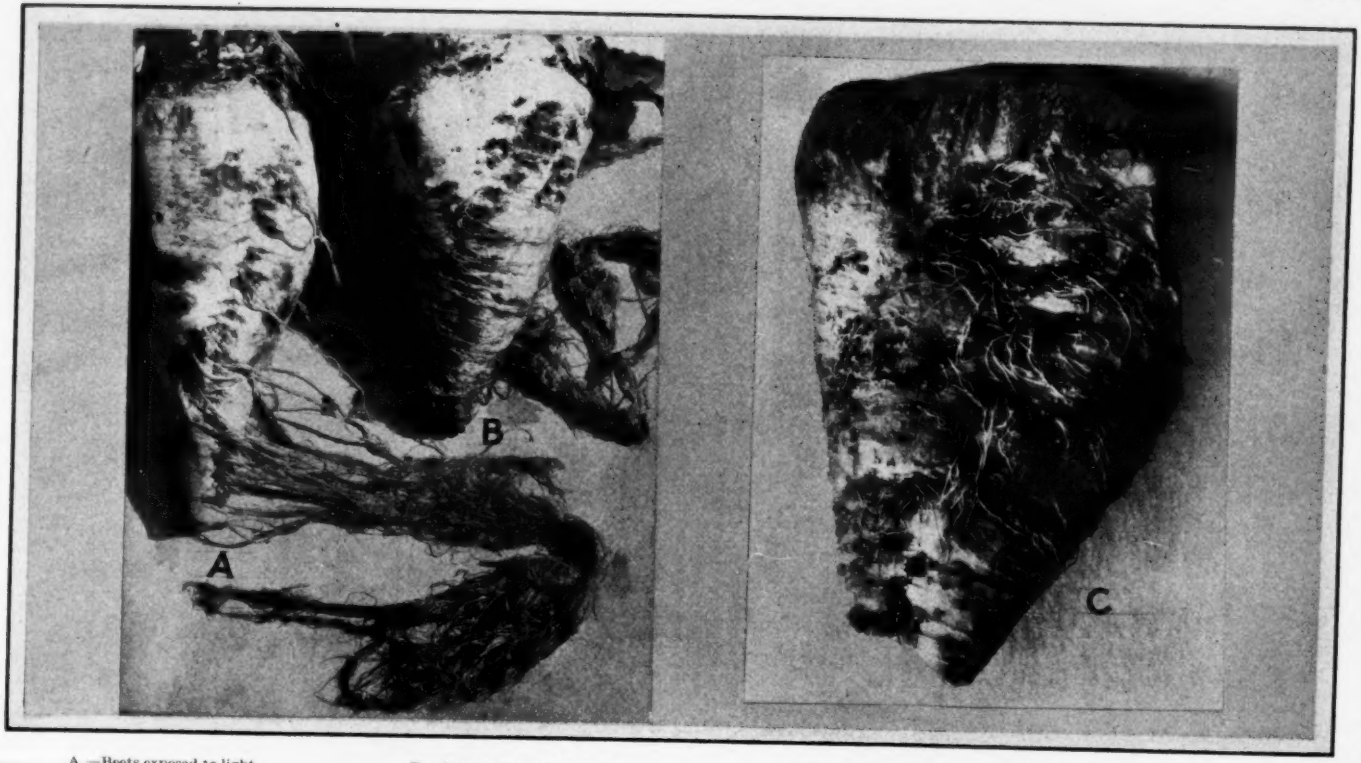


A.—Beets exposed to light

B.—Beets exposed to darkness

C.—Effect of removed crown on root-bud and side-root development

A.—Beets planted July 1 B.—Beets planted August 1 C.—Beets planted September 1



A.—Beets exposed to light

B.—Beets exposed to darkness

C.—Effect of removed crown on root-bud and side-root development

influence of minor factors can be considered. The best results are obtained when sound beets of good physical condition are planted early in the season. At this time the cold-air temperature inhibits seed-stalk and seed production and favors vegetative leaf production, while the cold-soil temperature is favorable for side-root production and food-mobilization processes in the beet. At this favorable soil temperature for side-root production the mother beet often shows extended enlargement with an abundant formation of new roots. This extended root system is necessary to supply water for the transpiration stream during the seed production period. In early spring the roots probably absorb limiting food materials from the soil which aid materially in the further development of the beets and especially the seed, as it is understood that the amount of available food materials in the soil varies considerably during the season. There is a further accumulation of available food materials in the beet by the digestion of the stored sucrose. These progressive changes are also accompanied by a vegetative leaf production, which undoubtedly aids in the accumulation of new food materials. As the season advances, the temperature of the soil rises to a point which is unfavorable for root production, while the temperature of the air rises to the optimum for seed-stalk and seed production. Under these conditions maximum seed production occurs, because the beet has an abundance of available food and absorption organs provided by the earlier development.

On the other hand, if beets are planted during midsummer, when the temperature of the soil is inhibitive to root growth and the temperature of the air is favorable for seed production, the beets are forced to immediate reproduction or death, and there is very little or no seed produced. In this case, all conditions are similar and favorable for maximum seed production, except that the beet has not previously passed through a period of food mobilization and vegetative development which is necessary to sustain the reproductive development.

SUMMARY

These investigations indicate that the second year's development of the mother beet should be divided into two distinct periods: The first period being characterized by food mobilization, vegetative foliage development, new root production, and absorption of soil nutrients; the second period by rapid utilization of mobilized food, development of seed stalks, and production of seed.

The optimum temperature for seed-stalk and seed development is higher than the optimum temperature for vegetative leaf and root development.

The cold air and soil temperatures during the first period inhibit the reproductive development, while these temperatures favor complete vegetative development.

The higher temperatures of the second period favor rapid reproductive development and result in the retarding of the vegetative development.

There is maximum reproduction when a plant passes through a first period of complete vegetative development in which the reproductive development is inhibited, followed by a second period of forced reproductive development in which the vegetative development is largely inhibited.

The first period is an essential prerequisite to the second.

The seed produced during the second period is directly proportional to the extent of development of the beet during the first period.

THE SOIL MULCH IN THE ABSORPTION AND RETENTION OF MOISTURE [1]

By M. A. McCall [2]

Dry Land Specialist, Washington Agricultiural Experiment Station

INTRODUCTION

Without irrigation successful crop production over a large part of the western United States is dependent on moisture stored in the soil. In part, this is due to limitations in total precipitation, to its seasonal distribution, or to a combination of the two; but, in any case, the factors affecting the control of soil moisture are of the greatest practical importance.

At one time the soil mulch was assumed to be the basis of all moisture control. It was regarded as essential, both in effective absorption and in retention. More recently, however, the mulch and its necessity seem matters of doubt, and while no one advocates the discontinuance of mulch-forming tillage, former ideas at least seem to be considered untenable. In discarding former theory, however, no general principle has been developed in its stead, and so far as more than local application is concerned, the situation is somewhat hazy. As indicative of the present somewhat contradictory state of affairs, the following may be noted:

REVIEW OF LITERATURE

Barker (*4*) [3] found that at Lincoln, Nebr., the loss of water due to direct evaporation from the soil was a small factor after the water became thoroughly distributed in the soil. Young (*26*), for the same conditions, concluded that a loose soil mulch was not much more effective than an unmulched soil in retarding the evaporation of moisture already established in the soil. Burr (*8*), working at North Platte, Nebr., when starting with a soil almost filled with water, and during a season of heavy rainfall, arrived at a conclusion similar to that of Young. For a less favorable season, and with a soil comparatively dry below the second foot, Burr states that "from the viewpoint of storing water a cultivated surface is essential."

Atkinson, Buckman, and Gieseker (*3*) show that summer tillage saved moisture in excess of that retained in a soil not tilled, but no direct statement is made that the benefit of the tillage was due either to the mulch effect or to killing weeds.

Call and Sewell (*9*), after a careful study of the moisture content of a mulched and an unmulched soil, conclude that under Kansas conditions a cultivated soil is no more effective than a bare uncultivated one in preventing evaporation; and in addition conclude (*10*) that "in the past too much emphasis may have been placed on tillage as a direct means of conserving moisture and liberating plant food and too little on it for the purpose of destroying weeds." After a rather extensive review of literature from a variety of sources, Sewell (*22*) emphasizes this latter conclusion.

Merrill (*21*) quotes Farrell as reporting certain experiments at Nephi, Utah, in which fall plowing did not give beneficial results in moisture conservation; but further quotes Widtsoe as concluding from other data that "fall plowing undoubtedly conserves the winter precipitation."

Cardon (*11*) shows that at Nephi, Utah, during each of the four years 1909 to 1912, inclusive, fall-plowed soil contained less moisture than that not plowed until spring.

Harris and Jones (*16*), reporting on the same and additional data as that of Cardon, show graphically a similar result. In addition, they show that in the fall an unmulched soil contained practically as much moisture as did a mulched one. They conclude that weed removal is the most marked benefit of tillage.

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[2] The author is now Agronomist in Charge of Cereal Agronomy, Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] Reference is made by number (italic) to "Literature cited," p. 831.

Harris and Yao (*18*) state that "moisture samples taken from the Nephi substation to a depth of six feet prove in every case that fall plowing preserves more moisture than spring plowing," a statement rather difficult to understand in the light of the statements of Cardon (*11*) and Harris and Jones (*16*).

Cates and Cox (*12*), basing their conclusions on data secured in studies at widely separated points over the entire United States, conclude that weed removal is the most important function of cultivation in handling the corn crop, and that the amount of moisture saved by such cultivation is negligible beyond a possible slight checking of run-off from heavy rains.

Fortier (*13, 14*) shows that mulching a soil after irrigation conserves moisture, and that the deeper the mulch up to 9 inches, the deepest used in his experiments, the more efficient the retention. At Wenatchee, Wash. (*14*), in a 21-day period during June, 1908, an unmulched soil lost 14.38 per cent, one with a 3-inch mulch, 3.98 per cent, one with a 6-inch mulch, 2.10 per cent, and one with a 9-inch mulch, 1.06 per cent of the water applied in a 6-inch irrigation. Willard and Humbert (*25*) show a similar result.

Harris and Bracken (*15*), from data secured under irrigation, state that cultivation is slightly more efficient in saving moisture than is no cultivation with weeds removed by pulling. They state that a comparison of deeply cultivated plats with those given shallow cultivation or no cultivation shows a decided advantage for the latter in moisture content at the lower soil depths.

McCall and Holtz (*19*) show that soils mulched during the period when moisture absorption was most active contained less moisture at the end of the fallow period than did other soils not mulched during the same absorption period. They conclude that the mulch probably aids in retaining moisture already in the soil.

Mathews (*20*), in reporting on the storage of moisture and its utilization by spring wheat, states that, as an average for all stations of the Office of Dry-Land Agriculture Investigations, Bureau of Plant Industry, United States Department of Agriculture, there are no notable differences in the moisture conserved by two continuously cropped plats, the one being fall plowed and the other spring plowed. He mentions certain seasonal conditions that may favor the first in the southern Great Plains, and the second, to the north, but that there are no consistent advantages in either case.

Shutt (*23*), in Canada, reports that a packed fallow contained more moisture than one not packed. This indicates either superior absorption or superior retention on the part of the firmly packed soil, although which is the more likely hypothesis is not suggested by the author.

From the foregoing it is clear that all investigators have not arrived at the same conclusion. It is equally clear that all are not measuring the mulch from the same point of view. In the conservation of natural precipitation the mulch must be considered both in its effect on absorption and its effect on retention. Where the mulch is measured solely as a retentive agent, as under irrigation, the evidence is unanimous that its effect is positive in saving moisture. In measuring the value of the mulch in relation to natural precipitation, where both considerations come into play, the result is less clear and the advantages, if any, are slight, neutral, or negative.

INVESTIGATIONS

Soil-moisture problems are a very important part of the program of the Adams Branch Station of the Washington Agricultural Experiment Station at Lind, Wash. Production in the territory this station serves is based on the summer-fallow system of alternate years of crop and fallow. One of the objects of the fallow is to conserve moisture and to carry it from one season to the next. As one of the factors affecting moisture conservation, the soil mulch has been studied during the six years 1918 to 1923, inclusive.

Under field conditions it is often difficult to differentiate between the two different relationships of the mulch. This is particularly true when the period of greatest precipitation is concurrent with that of greatest evaporation, as, for instance, in the Great Plains area. Climatic conditions on the Adams Station, however, meet the requirements for differentiation to a considerable degree. The distribution of rainfall, and the seasonal fluctuation of temperature and relative humidity (figs. 1, 2, and 3), divide the year into distinct periods, during any one of which either absorption or retention becomes most prominent. During the late fall, winter, and early spring, when a good proportion of all precipitation occurs, relative humidity is comparatively high and absorption is the most important consideration. During the summer temperatures are

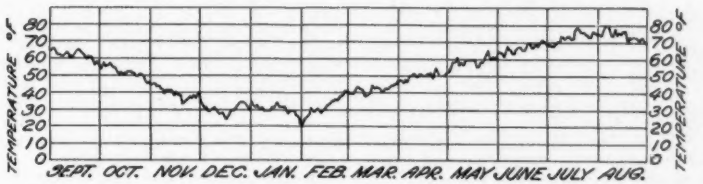

FIG. 1.—Mean daily temperature, based on an 11-year average, at Lind, Wash. During the period from November 15 to March 15, when mean temperatures are comparatively low, evaporation is less active than at any other time, and the opportunity for precipitation to be absorbed by the soil is correspondingly greater. The general relationship between mean temperature and rate of evaporation is shown in Figure 2

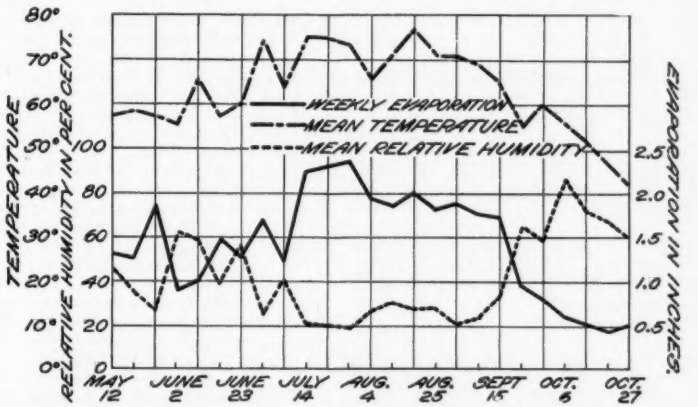

FIG. 2.—Relation between mean temperature, mean relative humidity, and total evaporation from a free water surface, by weeks, at Lind, Wash., May 9 to October 30, 1924. The dates given represent weekly centers

high, relative humidity is low, and little or no rainfall occurs. Retention then becomes paramount. The fallow period, from a moisture standpoint, extends from the removal of one crop to the beginning of the growing season of the next. Practically, on the Adams Station, it therefore consists of a first period of absorption, extending through the first fall, winter, and spring, followed during the summer by a period in which retention is important, and this, in turn, followed by a second period of absorption. The soil moisture content at the beginning and end of either of these periods should, therefore, give a basis for reliably measuring the effect of the mulch in whichever function is most important for that period.

The soil of the Adams Station is a very fine sandy loam of the Ritzville series. It is a typical semiarid soil, deep and uniform. The mechanical analysis of the soil by feet to a depth of 4 feet is given in Table I.

TABLE I.—*Mechanical analysis, by feet, to a depth of 4 feet of the soil on the Adams Branch Station, Lind, Wash. (19).*

Soil section	Fine gravel	Coarse sand	Medium sand	Fine sand	Very fine sand	Silt and clay	Total
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
First foot	0.00	0.08	0.13	9.82	45.88	45.05	100.96
Second foot	.00	.08	.08	8.68	46.75	44.40	99.99
Third foot	.00	.11	.14	8.04	52.01	39.70	100.00
Fourth foot	.00	.06	.05	6.10	50.24	43.45	99.90

The study of the mulch was made on the plats of the summer-fallow experiments. There are two series of these plats, one series being in crop and one in fallow each year. The plats are one-twentieth of an acre in area, 1 rod wide by 8 rods long.

Samples for moisture determinations were taken by foot sections to a depth of 4 feet, this depth being sufficient during the period of somewhat deficient moisture covered by the study.

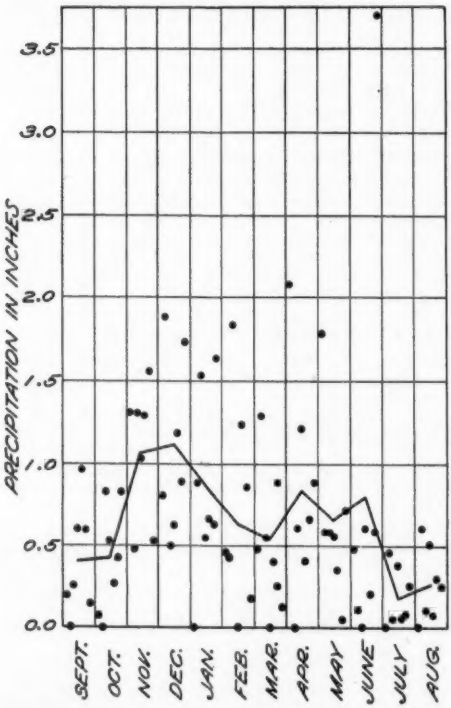

FIG. 3.—Precipitation by months, at Lind, Wash., September 1, 1916, to August 31, 1923. The tendency for greater precipitation during the winter months is apparent, as well as the unusual rainfall of June, 1923

Methods of sampling and of determining soil moisture are given in Bulletin 164 of the Washington Agricultural Experiment Station (*19*).

Moisture data are given in acre-inches, rather than in per cent of moisture-free soil. The volume weight of the surface 6 inches of a plowed soil is materially different from that of the same soil in an undisturbed condition, and the same per cent moisture content does not signify the same amount of moisture present in the same effective depth in the two cases. In the soil on which this study was made the volume weight of the plowed layer is approximately 60 per cent of that before stirring. The volume weight of the surface foot of such a plowed soil is 80 per cent that of the unstirred. Moisture content of the surface foot, where necessary, was calculated on this basis.

In Table II are given total soil moisture data for certain tillage variations in preparing the fallow, which indicate the reaction of the mulch in absorption each year during the period 1918 to 1923, inclusive.

All of the tillage variations as given in Table II were maintained in a well-mulched condition during the summer period of retention. So far as this period and the succeeding period are concerned, the result for all should have been similar. The essential difference between the variations was in the presence or absence of a soil mulch during all or a part of the first period of absorption. The only plats on which no mulch existed at any time during this first period were those of the early spring plowing with no previous treatment. All of the others were mulched for varying periods, either with a light mulch created by disking in August immediately after harvesting the preceding crop, or with a heavier mulch created by plowing at some later date.

Not once during the 6-year period did any of the mulched plats equal the unmulched plats in final moisture content. In several instances the shallow mulch of the harvest-disked and spring-plowed plats was almost as efficient in saving moisture, but this efficiency was not due to equality in moisture absorption. This is shown by comparing the dry fall-plowing alone and the dry fall-plowing with previous harvest-disking. As far as absorption was concerned, these two treatments should have given an identical effect, the difference between the two being in the presence or absence of weed growth in the stubble in the interim between harvest and the date of dry fall plowing. The comparative result between these two treatments is not always the same, due to the fact that weeds were not always present in the stubble, but the

TABLE II.—*A comparison of total and conserved soil moisture in the soil to a depth of 4 feet after the year of fallow, for six variations in fallow preparation*

[The essential difference between these variations lies in the presence or absence of a soil mulch during all or a part of the first major period of moisture absorption. Adams Branch Station, Lind, Wash., 1918 to 1923, inclusive]

Tillage variations	Soil moisture in acre-inches to a depth of 4 feet after the year of fallow								
	1918	1919	1920	1921	1922	1923	Average	Conserved moisture [a]	Comparative amount of conserved moisture
Dry fall-plowed	3.35	4.97	4.89	5.07	4.67	5.00	4.66	2.58	100
Harvest-disked, dry fall-plowed	4.34	5.34	4.76	4.82	5.48	5.03	4.96	2.88	111
Harvest-disked, wet fall-plowed	3.94	5.54	4.58	5.05	5.24	4.76	4.85	2.77	107
Harvest-disked, early spring-plowed	4.61	5.63	4.87	5.52	6.18	5.52	5.39	3.31	128
Wet fall-plowed	3.75	5.49	4.80	5.04	5.18	5.57	4.97	2.89	112
Early spring-plowed	4.70	5.98	5.32	5.76	6.47	5.60	5.64	3.56	138

[a] Residual moisture, 2.08 acre-inches to a depth of 4 feet, average of four seasons.

data indicate that on the average there was some saving of moisture through weed removal by harvest-disking. Despite this saving, however, the effect on absorption more than counterbalanced the gain from killing weeds, as shown by the slightly lower average moisture content of the harvest disking and early spring plowing, when compared with spring plowing with no previous treatment. A comparison of harvest-disking and wet fall-plowing with wet fall-plowing alone shows the same effect, due to the same cause.

It might be suggested that the moisture advantage of the spring-plowed soil was due entirely to the holding of snow by stubble. This factor undoubtedly is of some importance under certain conditions, but, so far as this experiment was concerned, it was relatively unimportant in determining total moisture. The effect of the mulch on absorption played a much larger part in the final result. This is indicated by comparing the moisture content of the harvest-disking and spring-plowing with that of the harvest-disking and dry fall-plowing. Stubble was not extensive on any of the plats, and as a result of the tillage, neither of these variations carried a stubble cover during the period of snowfall. The lower average moisture content of the second must be attributed, therefore, to a more pronounced inhibitory effect due to the deeper mulch of the dry plowing as compared with that of the disking. This indicates that, within certain limits, the inhibitory effect is proportional to depth in the mulch. That the effect of the mulch on absorption is not due to the physical condition resulting from tilling this particular soil when dry is shown by the fact that the mulch created by the wet fall-plowing also reacted in inhibiting absorption.

It is apparent, therefore, that during each of the six fallow periods covered by these data, soil covered by a mulch during all or a part of the first period of absorption contained less moisture at the end of the fallow than where no mulch existed during the same period of absorption. This indicates that the lower moisture content was due to an inhibiting effect of the mulch on absorption.

ABSORPTION

Detailed data on absorption are available for the seasons of 1920 to 1923, inclusive, and on retention for the seasons of 1921 to 1923, inclusive. In the study of absorption certain plats of the experiments, mulched and unmulched, were sampled at the beginning and at the end of each major absorption period. In the study of retention two plats were given no surface tillage whatever, but were kept free of growth by shaving with a sharp hoe. Any loose soil was removed by winds, and the surface of these plats was as free from both weeds and mulch as could be desired. The moisture content of these plats at the beginning and the end of the retention period was compared with that of other plats mulched during the same period. The data on absorption (Table III) will now be considered, followed by the results on retention (Table IV).

In the spring of 1921 the unmulched soil had absorbed 3.53 inches of water per acre. This was approximately 67 per cent of the total precipitation

TABLE III.—*Soil-moisture content in acre-inches by foot sections to a depth of 4 feet in mulched and unmulched soils, at the beginning and at the end of the first absorption period of the fallow, together with gains in moisture during the absorption period, Adams Branch Station, Lind, Wash., 1920 to 1923, inclusive*

Soil section	1920–1921						1921–1922					
	Mulched, fall plowed			Unmulched, stubble			Mulched, fall disked			Unmulched, stubble		
	Aug. 16, 1920	Apr. 14, 1921	Gain	Aug. 16, 1920	Apr. 14, 1921	Gain	Aug. 10, 1921	Apr. 7, 1922	Gain	Aug. 10, 1921	Apr. 7, 1922	Gain
First foot	0.24	1.52	1.28	0.24	1.76	1.52	0.28	1.70	1.42	0.27	2.09	1.82
Second foot	.49	1.65	1.16	.49	1.66	1.17	.56	1.42	.86	.52	1.59	1.07
Third foot	.56	1.14	.58	.57	1.32	.75	.64	.69	.05	.63	.75	.12
Fourth foot	.57	.61	.04	.57	.66	.09	.60	.65	.05	.63	.72	.09
Total gain			3.06			3.53			2.38			3.10
Gain below first foot			1.78			2.01			.96			1.28
Rainfall during interval, in inches			5.26			5.26			5.30			5.30

Soil section	1922–1923						3-year average					
	Mulched, fall plowed			Unmulched, stubble			Mulched			Unmulched		
	Sept. 7, 1922	Mar. 23, 1923	Gain	Sept. 7, 1922	Mar. 23, 1923	Gain	Fall	Spring	Gain	Fall	Spring	Gain
First foot	0.26	1.21	0.95	0.25	1.63	1.38	0.26	1.48	1.22	0.25	1.83	1.58
Second foot	.54	1.15	.61	.52	1.57	1.05	.53	1.41	.88	.51	1.61	1.10
Third foot	.61	.60	.01	.64	1.04	.40	.60	.81	.21	.61	1.04	.43
Fourth foot	.57	.60	.03	.63	.49	.14	.58	.62	.04	.61	.62	.01
Total gain			1.58			2.69			2.35			3.12
Gain below first foot			.63			1.31			1.13			1.54
Rainfall during interval, in inches			5.08			5.08			5.21			5.21

that had fallen between the dates of sampling. The mulched soil had absorbed 3.06 inches during the same period, or 58 per cent of the total precipitation. Depth of absorption is an important factor in subsequent retention. In the unmulched soil 2.01 inches of moisture had penetrated below the first foot, while in the mulched soil 1.78 inches had reached this same lower level. The unmulched soil contained 12 per cent more moisture in the area where it could be most effectively retained.

In the spring of 1922 the soil not mulched during the preceding winter had absorbed 3.10 inches of moisture, and of this amount 1.28 inches was below the first foot. The mulched soil in the same time had absorbed 2.38 inches, with 0.96 inch below a depth of 1 foot. During this period the unmulched and mulched soils absorbed and retained, respectively, 58 per cent and 45 per cent of the total precipitation. In total absorption, the unmulched soil was 30 per cent superior to the mulched, and in the amount penetrating below the first foot was 22 per cent superior.

In the spring of 1923 the data show that the unmulched soil had absorbed 2.69 inches of moisture in excess of that present the previous fall. Of this amount, 1.31 inches was below the first foot. The mulched soil absorbed 1.58 inches, with only 0.63 inch at the lower levels. The data for the spring sampling of the unmulched soil indicate that a loss occurred in the fourth foot of this treatment after soil moisture was determined the previous fall. This loss was due to weed growth (Russian thistle, *Salsola kali tenuifolia* G. F. W. Mey) subsequent to the date of dry fall-plowing, and after the moisture samplings were made. Despite this moisture loss from the unmulched soil above that occurring from the mulched treatment, absorption was sufficiently greater where the mulch was absent to entirely recover this loss and with substantial additions in all sections except in the fourth foot. Total absorption in the unmulched

soil was actually the absorption shown by the data, plus the undetermined loss. On the basis of the data, the unmulched soil absorbed 53 per cent and the mulched soil absorbed 31 per cent of the total intervening precipitation. The unmulched soil was 69 per cent superior in total absorption and slightly over 200 per cent superior in the amount of moisture below the first foot.

In every instance the unmulched soil was superior as an absorbing agent. The comparative efficiency of the mulched and unmulched soils in absorption is shown graphically in Figure 4.

In 1922 the loss from the mulched soil amounted to 0.71 inch, and, considering the gain in the lower 2 feet, this loss came entirely from the first foot. The unmulched soil sustained a loss of 1.61 inches, and of this loss 0.21 inch came from below the first foot. Neither the mulched nor the unmulched soil lost moisture from the third foot in 1922.

In 1923 the mulched soil showed a total loss from July 6 to September 20 of 0.49 inch. Of the total loss, but 0.04 inch came from below the first foot, and none was lost from the third foot by evaporation. The unmulched soil in the same period lost 0.95 inch

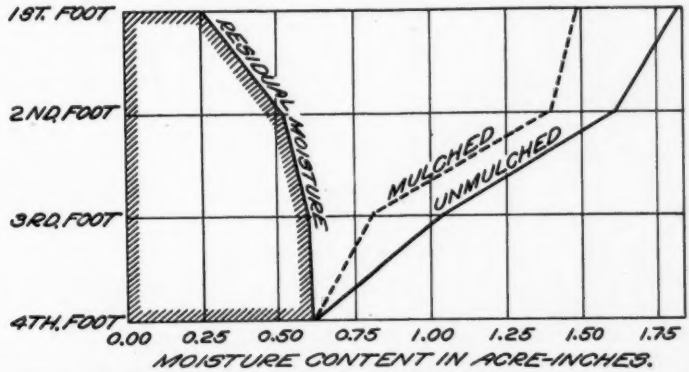

FIG. 4.—Average moisture content in acre-inches of mulched and unmulched soils at the end of the first period of moisture absorption during the fallow year, Adams Branch Station, Lind, Wash., 1920 to 1923, inclusive

RETENTION

Data showing the relation of the mulch to retention are given in Table IV. These data show that during the summer of 1921 the soil covered by a 6-inch soil mulch lost 1.02 inches of moisture. Of this total loss by evaporation, 0.12 inch came from below the first foot. In the lower 2 feet of this soil there was a gain by downward movement of 0.27 inch. The soil covered by a 4-inch mulch lost a total of 1.59 inches of moisture, or 56 per cent more than the 6-inch mulched soil. Of this amount 0.55 inch came from below the first foot, and 0.10 inch came from the third foot. The unmulched soil lost 2.02 inches of moisture. This was 198 per cent of that lost from the 6-inch mulch. Of the total lost from the unmulched soil, 0.85 inch came from below the first foot, and 0.32 inch came from the third foot.

of moisture, with 0.32 inch coming from below the first foot. A very small loss by evaporation was indicated from the third foot of the unmulched soil.

The data for retention show the mulch to have a positive effect in conserving moisture, when considered only from that standpoint, and that efficiency in retention is increased by depth in the mulch. The comparative efficiency of the mulch in retention is shown in Figure 5.

CONCURRENT ABSORPTION AND RETENTION

Ordinarily, on the Adams Station, the mulch need be considered only as a retentive agent during the summer season, but in June, 1923, a rainfall of 3.69 inches gave an opportunity to observe it as an absorbing agent under such conditions also. In effect, the mulch was functioning in absorption and retention at one and the same time (Table V).

TABLE IV.—*The soil-moisture content in acre-inches by foot sections to a depth of 4 feet, in mulched and unmulched soils, at the beginning and at the end of the period of extreme summer evaporation during the year of fallow, together with moisture losses occurring during the period, Adams Branch Station, Lind, Wash., 1921 to 1923, inclusive*

Soil section	1921									1922					
	6-inch mulch			4-inch mulch			Unmulched			Mulched			Unmulched		
	Apr. 14	Sept. 3	Loss	Apr. 14	Sept. 3	Loss	Apr. 14	Sept. 3	Loss	Apr. 7	Sept. 6	Loss	Apr. 7	Sept. 6	Loss
First foot	1.63	0.73	0.90	1.76	0.72	1.04	1.66	0.49	1.17	1.70	0.81	0.89	2.09	0.69	1.40
Second foot	1.65	1.26	.39	1.66	1.21	.45	1.56	1.03	.53	1.42	1.28	.14	1.59	1.15	.44
Third foot	1.18	1.18	.00	1.32	1.08	.24	1.41	.98	.43	.69	.96	*.27	.75	.95	*.20
Fourth foot	.62	.89	*.27	.66	.80	*.14	.61	.72	*.11	.65	.70	*.05	.72	.75	*.03
Total loss			1.02			1.59			2.02			.71			1.61
Loss below first foot			.12			.55			.85			.18			.21
Loss from third foot by upward movement			.00			.10			.32			.00			.00
Gain in fourth foot			*.27			*.14			*.11			*.05			*.03

Soil section	1923						3-year average					
	Mulched			Unmulched			Mulched			Unmulched		
	July 6	Sept. 20	Loss.	July 6	Sept. 20	Loss	Spring	Fall	Loss	Spring	Fall	Loss
First foot	1.34	0.89	0.45	1.42	0.79	0.63	1.56	0.81	0.75	1.72	0.66	1.06
Second foot	1.74	1.39	.35	1.51	1.21	.30	1.60	1.31	.29	1.55	1.13	.42
Third foot	1.41	1.33	.08	1.29	1.17	.12	1.99	1.16	*.07	1.15	1.03	.12
Fourth foot	.72	1.11	*.39	1.11	1.21	*.10	.66	.90	*.24	.81	.89	*.08
Total loss			.49			.95			.73			1.52
Loss below first foot			.04			.32			.02			.46
Loss from third foot by upward movement			.00			.02			.00			.04
Gain in fourth foot			*.39			*.10			*.24			*.08

* Gain.

TABLE V.—*Soil moisture content in acre-inches to a depth of 4 feet of a mulched and an unmulched soil at the beginning and at the end of a period of summer precipitation, Adams Branch Station, Lind, Wash., 1923*

Soil section	Mulched			Unmulched		
	May 31	July 6	Gain	May 31	July 6	Gain
First foot	1.03	1.34	0.31	1.48	1.42	−0.06
Second foot	1.41	1.74	0.33	1.32	1.51	0.19
Third foot	1.12	1.41	0.29	1.15	1.29	0.14
Fourth foot	0.59	0.72	0.13	1.01	1.11	0.10
Total gain			1.06			0.37
Gain below first foot			0.75			0.43
Rainfall during interval, in inches			3.69			3.69

Of the total rainfall of 3.69 inches occurring between May 31 and July 6, 1923, the mulched soil absorbed 1.06 inches. This was 28.7 per cent of the entire amount. Of the total amount absorbed, reference to Table IV shows that practically one-half was lost by evaporation during the remainder of the summer, as well as such limited additional rain as fell in the interim. By September 20 only 15.4 per cent of the entire June rainfall remained in the mulched soil. The unmulched soil had absorbed and retained only 0.37 inch of the June rainfall on July 6, or 10 per cent of the total. By September 20, even this small amount had been lost, as well as an additional 0.58 inch from that previously in the soil.

In a general way, the conditions prevailing during this period and the final result were both very similar to those characteristic of the Great Plains area, and their proper interpretation should give a basis for understanding the somewhat divergent data from this region and that west of the Rocky Mountains.

DISCUSSION

In most instances data have not been reported by other investigators in a form lending itself to a true evaluation of the mulch. The data from the Nephi (Utah) Substation, as reported by Cardon (*11*) and by Harris and Jones (*16*), the two being in substantial agreement, do, however, lend themselves to a measurement of the absorptive effect. Data after Cardon are shown graphically in Figure 6.

Regarding these data, Cardon (*11*) says:

"The facts thus brought out seem to indicate that at Nephi stubble land allows the winter precipitation to penetrate to greater depths than fall-

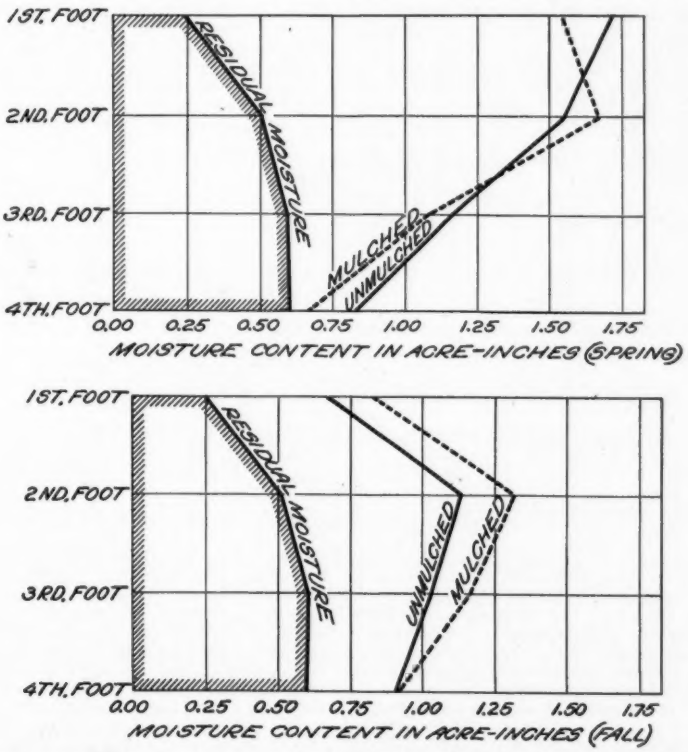

FIG. 5.—Average moisture content in acre-inches of mulched and unmulched soils in the spring and in the fall of the fallow year, Adams Branch Station, Lind, Wash., 1920 to 1923, inclusive

plowed land, and that the loose surface of the fall-plowed land retains more of the precipitation of winter than the compact surface of the stubble land."

Cardon recognized that fall plowing hindered moisture absorption, although he did not directly attribute the effect to the mulch as an inhibiting agent.

The various data presented having shown the mulch to be an inhibitory agent in absorption and a positive agent in retention, the fundamental reasons for these reactions merit attention.

On the Adams Station the prevailing type of rainfall is characteristically in the form of light, intermittent showers. Very rarely does a single rain total 0.50 inch, and the majority of individual showers amount to 0.30 inch or less. Cardon (*11*) says:

"Most of the rainstorms at Nephi have been small and generally almost negligible." This type of rainfall undoubtedly is a factor in the inhibitory reaction of the mulch.

When sampling, the surface 6 inches of a mulched and an unmulched soil immediately after a rain, the moisture content of the mulched soil on a percentage basis is always greater, the loose soil having more moisture in proportion to soil in the surface area. This fact was noted by Cardon (*11*) and by Harris and Jones (*16*).

Widtsoe and McLaughlin (*24*) and Fortier (*13*) have called attention to the fact that evaporation is always greater from a soil the greater the percentage of moisture present, or, as stated by the former, "the rate of loss of water from a soil increases as the initial per cent of water in the soil increases." This means that drying winds or other factors favoring evaporation immediately following a rain will more quickly remove moisture from the loose soil of a mulched surface than from the more firm soil in an undisturbed condition. After an intervening period of evaporation, a second shower falling on the two soils, because of the larger amount of moisture remaining in the unstirred soil, is absorbed and conducted more deeply into the unstirred soil than where the mulch is present. Alway and McDole (*2*) have shown that depth of penetration increases in direct proportion to initial moisture content, as has also been noted by Burr (*8*) and by Buckingham (*7*). Such deeper penetration, following a series of showers with intervening periods of evaporation, gives the unstirred soil its final moisture advantage, the result being due to the cumulative effect of intermittent rainfall and intervening evaporation, rather than to original superior penetration in the more firm soil. A light, intermittent rainfall contributes to the result, since heavy showers, by wetting entirely through the mulch, destroy the mulch effect, the mulch in such cases being an actual benefit in so far as it prevents surface run-off.

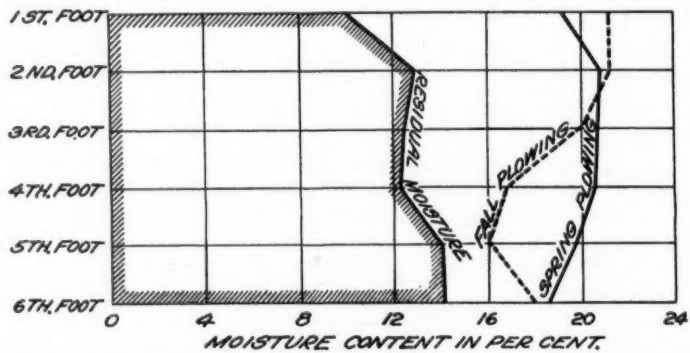

FIG. 6.—Average moisture content in per cent of moisture-free soil of fall-plowed and spring-plowed soils at the end of the first winter of the fallow, Nephi, Utah, 1909 to 1912, inclusive

Bouyoucos (*6*) has shown that when freezing occurs in soils not saturated, there is a withdrawal of soil water from the finer capillaries into the larger pore spaces. Dry-farm soils are seldom so saturated that the familiar heaving effect of more humid regions occurs, but it is possible that there might occur in freezing a certain withdrawal of moisture from the more firm soil below into the larger interspaces of a loose mulch. With subsequent thawing, a larger proportion of moisture would thus be left

near the surface exposed to evaporation. This factor might have a bearing on increasing the inhibitory effect of the mulch on absorption during the colder seasons of the year, but if so it has been less important on the Adams Station than the other factors outlined above, as evidenced by the fact that the inhibitory effect has been apparent before any freezing weather occurred.

That the inhibitory effect of the mulch on absorption tends to be proportional to the depth of the mulch was brought out by discussion of the data in Table II. The deeper mulch of the dry fall plowing was a more effective inhibiting agent than the more shallow mulch of the disking. Any condition which tends to retain the largest percentage of current showers near the surface undoubtedly would aggravate the inhibitory effect of the mulch on absorption, and increased depth in the mulch has such an effect. Under normal field conditions a stirred soil tends naturally to settle as it is wet, and a deeper mulch, requiring a larger amount of moisture to entirely wet through it, will naturally remain an effective inhibiting agent for a longer time than would one more shallow. The volume and frequency of individual rains naturally have a very important bearing on this factor.

In no instance has the writer been able to find a record of the mulch failing to conserve moisture when it is clearly shown that it was confined to that single function. The data presented in this study for the summer periods are, therefore, in complete agreement with the findings of others so far as this one phase is concerned.

In general, the theoretical operation of the soil mulch in preventing the loss of soil moisture through evaporation might seem too well known to require an extended discussion; yet, because the issue has been confused in some cases, a certain elaboration is desirable. It has generally been considered as established beyond controversy that soil moisture moves within the soil in response to the forces of capillarity. In accordance with this concept, as evaporation removes moisture from the surface soil, other moisture moves up from more moist underlying soil, and being in turn removed by evaporation, a more or less continuous movement from below to the surface is established. The stirring, incident to creating the soil mulch, decreases the number of points of capillary contact, and, by exposing additional surface to evaporating agencies, increases evaporation in the immediate surface soil to a rate beyond the ability of capillary movement to replace losses. As a result, the surface soil becomes dry, and being already loosened by the stirring, the mulch effect is created. Buckingham (*7*), Alway and Clark (*1*), and others have shown that such a dry soil retards capillary movement, hence, when present as a loose surface layer, necessarily would check evaporation.

In introducing the review of literature in their discussion of the soil mulch, Call and Sewell (*9*) make the statement that "capillary movement without the presence of a water table (with a dry subsoil) has not been demonstrated." From the data presented in Table IV, it is very evident that moisture withdrawal, and hence movement, occurred in both the mulched and unmulched soils. This movement was both upward and downward, but despite the fact that downward movement, aided by gravity, tends to be greater than that in an upward direction, as noted by Harris and Turpin (*17*), this latter was slight in the unmulched soil, and, varying with season, there occurred a withdrawal of moisture extending into the third foot. How much deeper this upward movement might or might not have extended with greater moisture content and deeper penetration is, of course, a matter of conjecture, yet it was extensive enough in any case to demonstrate that upward movement occurs without a water table. That this was a capillary movement seems the logical conclusion, since Buckingham (*7*) and Bouyoucos (*5*) both have shown that internal evaporation and diffusion have very little effect on moisture movement within the soil.

It is well known that the rapid evaporation of arid regions often greatly exceeds the ability of any soil to conduct moisture to the surface by capillarity, resulting in a naturally dry surface condition, this dry surface acting in preventing further loss of moisture as a "natural mulch" (*7*). Many have felt that such a natural mulch might make unnecessary the artificially created one, and that benefits have been ascribed to the latter which may, in reality, be just as effectively secured by natural means and without unnecessary labor. The data presented, however, would not indicate the "natural mulch" to be equal to the artificially created one on the Adams Station, and, eliminating concurrent precipitation and its effects on soil moisture content in the mulched and unmulched soil, it seems very doubtful

if the "natural mulch" is ever equal in efficiency to the artificial in the actual retention of moisture already in the soil.

Since there is, therefore, a proved upward movement of moisture in the soil under conditions of rapid removal by evaporation, and since the data presented show that the soil mulch can and does check a certain part of this movement and loss, there can be little doubt that as a purely retentive agent, the soil mulch has a positive effect.

The two effects of the soil mulch in absorption and retention have been shown and their operation has been described. The data for the period of summer rainfall indicate how these two effects may be modified by type of rainfall and intensity of evaporation. In the particular June period considered in this study there were two individual rains, each greater than 1 inch in amount, and both of which penetrated well below the mulch. Following these rains evaporation was rather active. When volume of rainfall is great enough to penetrate the mulch and to establish connection with the underlying soil, the mulch no longer acts in absorption in the same manner as with lighter precipitation. If conditions favor rather intense evaporation, tillage given immediately after such rainfall, by renewing the mulch, may retain a certain part of the moisture which has penetrated below the mulched area. When the mulch is not reestablished after the rain, under such conditions there is greater loss of moisture from the unmulched soil. In conserving natural precipitation, the most important consideration is to reduce evaporation in the surface area to a point that moisture may have an opportunity to move downward into the lower soil. If the rate of evaporation is not extreme, and moisture falling on the surface is not removed, either as run-off or by evaporation, before it has an opportunity to penetrate to lower levels, there is nothing to be gained by creating a mulch, either before or after a period of rainfall. Light showers not sufficient to penetrate the mulch have little effect one way or the other on total moisture content, for either in a mulched or an unmulched soil such a limited amount of moisture near the surface is quickly removed if evaporation be active. The value of the soil mulch in conserving moisture in a region of summer rainfall, therefore, is entirely dependent on the volume of individual showers and on the intensity of succeeding evaporation. Practically this may result in little effect from the mulch one way or the other, as shown, for instance, by the data reported by Call and Sewell (*9*), by Barker (*4*), by Young (*26*), and others.

In the foregoing discussion there is no intent to convey the idea that the balance of the inhibitory effect of the mulch on absorption and its positive effect in retention is the sole determiner of the final result in tillage practice. Soil type, temperature, relative humidity, the volume, character and distribution of precipitation, etc., may give other factors prominence so that the presence or absence of the mulch is not always of equal importance. In districts of heavy snowfall and of comparatively high winds the protective effect of a heavy stubble in holding snow or in checking the evaporative influence of the wind may contribute very largely, for instance, to final soil moisture content; and in such a case all of the benefits can not be ascribed to the absence of a mulch that might hinder absorption. If precipitation is of such volume or character that the mulch functions for only a short time, there will be little effect, either one way or the other, from mulch-forming tillage. Whether evaporation is or is not active naturally has a decided influence on the result. These and other contributing factors must all be taken into account in regulating practice; yet, allowing to each factor its relative importance, the effect of the mulch as a mulch should still be given full consideration.

CONCLUSIONS

(1) The soil mulch has an inhibitory effect on moisture absorption, under conditions where individual rains are not of sufficient volume to fully penetrate the mulch.

(2) The mulch inhibits absorption by increasing the amount of current evaporation in the newly fallen moisture. The volume weight of the stirred mulch being less than that of an equal depth of unstirred soil, the moisture content of the mulched soil immediately after a rain is higher on a percentage basis. When conditions favor evaporation, the result is a greater total loss from the mulched soil. The final moisture content is due to a cumulative effect following several rains.

(3) The soil mulch prevents the loss of moisture already in the soil by checking evaporation.

(4) The practical use of the soil mulch in moisture conservation is

dependent on climatic conditions which influence the prominence of either the inhibitory effect on absorption or the positive effect on retention, or which may nullify either or both.

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FUNDAMENTALS FOR TAXONOMIC STUDIES OF FUSARIUM[1]

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INTRODUCTION

There has been a long-felt need for a general conference of all workers on the Fusarium problem in order that a uniform taxonomy may be formulated upon which future work can be based. The various workers have always been fundamentally in reasonably close agreement regarding the principles of Fusarium classification, but there has been a number of points which were not clearly understood, possibly because the investigators have not been sufficiently in contact with each other. Variations in the methods employed in different laboratories have complicated the problem of species identification. The fact that the fungi vary according to the environment, age, and source of culture and the failure of different workers to understand one another has, it is believed, largely accounted for the differences arising from Fusarium studies.

Recent investigations on the banana-wilt problem conducted by the United Fruit Co. in Central America showed the immediate necessity of a general conference on the Fusarium problem. Because of the large number of related Fusaria encountered in a study of the banana-wilt-producing organism on banana plantations, it was deemed advisable to complete the fungous investigations with a final study in Europe or in the United States. The proposed studies of the United Fruit Co. were discussed at the meeting of scientific societies in Cincinnati, in December, 1923, and through Dr. W. A. Orton a plan for an American conference on Fusarium was proposed. The idea involved the United Fruit Co.'s bringing Dr. H. W. Wollenweber over from Germany, and Dr. O. A. Reinking from Central America. The United States workers included Dr. C. D. Sherbakoff, of the University of Tennessee, Miss Helen Johann and Mrs. Alice A. Bailey, representing, respectively, the Offices of Cereal Investigations, of Cotton, Truck, and Forage Crop Disease Investigations, and of Pathological collections of the United States Department of Agriculture, in a joint study of Fusarium classification. The United Fruit Co., through its director of agricultural research, Dr. John R. Johnston, adopted this liberal policy in supporting the scientific work to a greater extent than their purely economic interests required. It was agreed, on the invitation of Dr. L. R. Jones, to hold the conference at the University of Wisconsin, in Madison.

The purpose of the conference was to give an opportunity to cooperate in a personal way, to compare cultures assembled from all possible sources, and in this manner to clear up the somewhat tangled taxonomy of this difficult genus. All the important European cultures, those being studied at present in the United States, and the collections made from the tropics, primarily from Central America, were assembled at the meeting for special study and comparison. The work of the conference covered, in so far as possible, the study, comparison, and identification of the specimens and cultures of the fungi then available. The main studies were made on the tropical collection, since it was agreed, because of the part taken by the

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[2] Engaged in special investigations for the United Fruit Co. during the Fusarium conference at Madison, Wis., in August, 1924.

[3] Present at the conference in cooperation with the Offices of Cotton, Truck, and Forage Crop Disease Investigations, of Cereal Investigations, and of Pathological Collections, Bureau of Plant Industry, U. S. Department of Agriculture.

[4] The writers are indebted to the Departments of Plant Pathology and of Botany of the University of Wisconsin for providing facilities with which to carry on the investigations during the period of the conference.

United Fruit Co., that the conference should facilitate, in the best way possible, a satisfactory definition of the numerous species found on banana plantations. The studies made of the entire collection embodied species from all sections of the genus Fusarium, including important border-line strains, thereby making it possible to arrive at a uniform taxonomy of the group. It is believed that the main points of difference have been agreed upon and that the present paper will present a clearer understanding of the Fusarium problem from the standpoint of the identification of species.

Material of each species studied and identified at the conference will be deposited in the pathological collections herbarium of the Bureau of Plant Industry, United States Department of Agriculture, for permanent preservation. The cultures will be prepared in the manner most satisfactory for the preservation of herbarium material. Pure cultures of each of the species studied and identified will also be placed in the bureau. These cultures will have substantially the status of type specimens.

CRITERIA OF THE NORM

Only a résumé of the criteria of the norm will be here considered, since a complete discussion of the subject has appeared in former publications (*5*, *9*).[5] The normal condition of Fusaria from the standpoint of determination may be present in nature, but generally it must be produced by growing the fungi under pure culture conditions. A few Fusaria such as *F. dimerum* Penz., *F. scirpi* Lamb. et Fautr., *F. culmorum* (W. G. Sm.) Sacc., and some others may be determined directly from the fungus growing under natural conditions without resorting to pure cultures. Other Fusaria, which appear primarily in a microconidial stage under natural conditions and produce only a few sickle-shaped spores, are more difficult to identify. For these the so-called normal growth must, at first, be produced. It is, therefore, necessary to make a careful study from pure culture in order accurately to identify these organisms. Generally, macroconidia are regarded as the normal spore type. In certain groups, however, the microconidia may have definite characters, such as a pearlike shape or a formation in chains, which aid in the determination of the section (Sporotrichiella, Arthrosporiella) and, in exceptional cases, may even lead to the identification of species (*F. poae* (Peck) Wr., *F. moniliforme* Sheld., *F. decemcellulare* Brick.).

Other normal reproductive stages, such as chlamydospores, by their presence or absence indicate the border of certain groups (Elegans, Lateritium). Sclerotia may be characteristic for groups (Lateritium) and even for a number of species (*F. sclerotium* Wr.). Color of the conidia and color of the aerial mycelium and stroma are further reliable characters for taxonomy. Minor characters such as mycelium, hyphae with specialized cells, coremia-like aggregations of hyphae, and aromatic odors may also be of some importance for differentiation. In opposition to the norm there are abnormal features which might be valuable to mention in the diagnosis. Not so much stress, however, is placed on degenerated conditions in the life cycle of Fusaria.

[5] Reference is made by number (italic) to "Literature cited," p. 843.

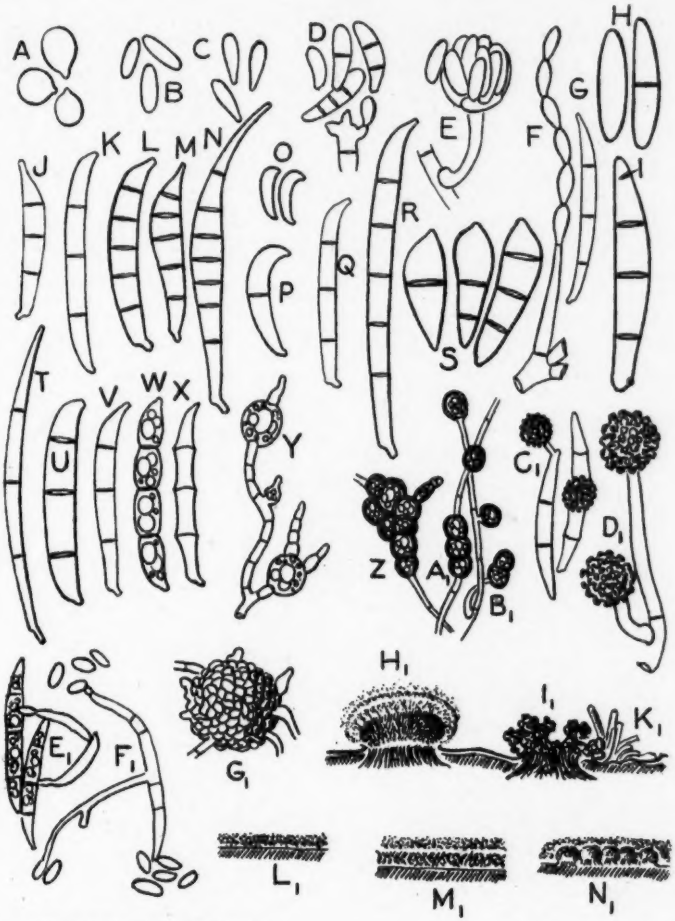

FIG. 1.—Stages in the life cycle of Fusaria:
A–F, H and S, microconidia.
I–R, T–X, macroconidia.
Y, Swellings of hyphae.
Z, A_1–D_1, Chlamydospores.
E_1–F_1, Conidial propagation from conidia.
G_1, Sclerotium.
H_1, Sporodochium.
I_1, Sclerotia in clusters on an erumpent base.
K_1, Columns formed by conidia borne in chains.
L_1–N_1, Pionnotes covering either the medium directly (L_1), or a mycelial sheet (M_1), or numerous associated sporodochia (N_1) formed on the substratum.

Examples: *Fusarium poae* (Peck) Wr. (A, J), *F. orthoceras* App. et Wr. (B, E, K, A_1, B_1), *F. moniliforme* Sheld. (C, F, G, G_1), *F. chenopodinum* (Thüm.) Sacc. (D, M, N, Z), *F. argillaceum* (Fr.) Sacc. (H, I, C_1, D_1), *F. subcarneum* Crouan (L), *F. flavum* (Fr.) Wr. (O), *F. dimerum* Penz. (P), *F. salicis* Fuck. (Q), *F. sarcochroum* (Desm.) Sacc. (R, S), *F. viticola* Thüm. (T), *F. solani* (Mart. pr. p.) App. et Wr. (U), *F. vasinfectum* Atk. (V, normal, W, swollen, X, dried spore), *F. tricinctum* (Cda.) Sacc. with swellings of hyphae (y), *F. macroxysporum* Lindf. with pionnotal propagation of macroconidia (E_1) and development of microconidia (F_1).

For a proper understanding of the terms (*8*) used for normal forms of reproductive stages and mycelium of Fusaria the characters considered of primary importance are enumerated below.

I. NORMAL REPRODUCTIVE STAGES

1. *Microconidia:* 0–(0–3)–septate, globose-pearshaped as in *F. poae* (Peck) Wr. (fig. 1A); ellipsoidal as in *F. orthoceras* App. et Wr. (fig. 1, B); ovoid-fusoid as in *F. moniliforme* Sheld. (fig. 1, C); comma-shaped as in *F. chenopodinum* (Thüm.) Sacc. (fig. 1, D). They may be found scattered (fig. 1, A–D), in false heads (fig. 1, E), or in chains (fig. 1, F) and may form sporodochia and pionnotes.

2. *Macroconidia*: 0-pluriseptate, dorsiventral, fusoid to sickle-shaped, apedicellate and pedicellate, scattered, sporodochial and pionnotal.

   a. Septation types:
      0–septate, *F. flavum* (Fr.) Wr. (fig. 1,O);
      1–septate, *F. dimerum* Penz. (fig. 1, P);
      3–septate, apedicellate, *F. betae* (Desm.) Sacc. (fig. 1, K);
      3–septate, pedicellate, *F. poae* (Peck) Wr. and (fig. 1, J);
      5–septate, *F. chenopodinum* (Thüm.) Sacc. and (fig. 1, M);
      5–7–septate, *F. chenopodinum* (Thüm.) Sacc. and (fig. 1, N);
      9–septate, *F. decemcellulare* Brick.

   b. Sporulation types: Scattered; in false heads; pionnotes, continuous, gelatinous, slimy, (*1*) formed on the naked surface of the substratum (fig. 1, L_1) as in section Eupionnotes, species of section Roseum, Gibbosum, Elegans and Discolor, or (*2*) on a mycelial sheet as in *F. avenaceum* (Fr.) Sacc. (fig. 1, M_1) or (*3*) composed of an aggregation of sporodochia as in *F. martii* App. et Wr. (fig. 1, N_1); sporodochia, (*1*) tuberculariallike (fig. 1, H_1) found in the majority of species of all sections except Ventricosum and some species of Elegans and Eupionnotes (*2*) in columns representing a chainlike development, as in *F. stilboides* Wr. (fig. 1, K_1).

   c. Shape: Fusoid-lanceolate, slightly curved, mostly apedicellate, *F. argillaceum* (Fr.) Sacc. (fig. 1, I); slightly sickle-shaped, apedicellate, *F. betae* (Desm.) Sacc. (fig. 1, K); sickle-shaped, pedicellate, most of the species; strongly dorsiventral, *F. scirpi* Lamb. et Fautr. and *F. chenopodinum* (Thüm.) Sacc. (fig. 1, N); terminal cell suddenly constricted, *F. culmorum* (W. G. Sm.) Sacc. and *F. chenopodinum* (Thüm.) Sacc. (fig. 1, M); terminal cell whiplike, much elongated *F. scirpi* Lamb. et Fautr. and *F. chenopodinum* (Thüm.) Sacc. (fig. 1, N); curvature within the limits of conical sections as elliptic (fig. 1, M), parabolic and hyperbolic (fig. 1, N). The curvature is mainly determined from the dorsal and ventral sides of spores, in side view, within the septation region.

3. *Chlamydospores:* Terminal (fig. 1, B_1, D_1), intercalary (fig. 1, A_1), single, in chains, in clusters, mycelial (fig. 1, Z to B_1), conidial (fig. 1, C_1).

   a. Terminal: In sections Elegans, Martiella, Ventricosum; unicellular, *F. argillaceum* (Fr.) Sacc. (fig. 1,N D_1); 0–1-septate, sections Martiella and Elegans (fig. 1, B_1). Most fungi forming terminal spores may also develop intercalary spores. In section Ventricosum no intercalary spores are produced.

   b. Intercalary: Mostly 0–1-septate (fig. 1, A_1) in sections Elegans, Martiella, Discolor, Gibbosum, and Eupionnotes (subsection Chlamydospora). Chains and clusters (fig. 1, Z) may also occur in most of these sections.

4. *Sclerotia:* Plectenchymatic structures, general appearance similar to perithecia of Gibberella, but with uniform structure throughout. Globose or rugose, single or in cauliflowerlike clusters (fig. 1, G_1). Color, blue in sections Roseum, Gibbosum, Lateritium, Discolor, Elegans, and Martiella; brownish to dark brown in Arthrosporiella (*F. diversisporum* Sherb.), Gibbosum, and some species of section Discolor.

II. Color characters

1. *Color of conidia:* Brownish white to golden yellow in section Martiella; brownish white to pale orange in section Elegans, Discolor, Gibbosum, and Arthrosporiella; orange in section Roseum Lateritium, Eupionnotes and Arachonites. The color of conidia is sufficiently constant to be a reliable character for some groups.
2. *Color of aerial mycelium:* White, rose to yellow and blue, mostly representing a diffused color of the stroma. Changes in color of mycelium due to the reaction of the substratum is substantially the same as that discussed under color of stroma.
3. *Color of stroma:* Brownish white, carmine, yellow, and blue. The acid modification is especially well developed on sterilized rice and may aid in the identification of Fusarium groups. This acid modification differs in some sections for it is golden yellow in most of the species in section Discolor, Saubinetii, Roseum, Sporotrichiella, and in some of Gibbosum; rose to vine red in many species of section Elegans; and rose to brown color, with a tendency toward diffusing into rice, in section Martiella. The basic color modification is mostly blue or violet. In general, all of these fungi produce on rice an acid color modification that may change gradually with age into an alkaline modification. This color change can be produced at once by the addition of sufficient alkali to a well-developed culture of the fungus on rice. Other characteristic colors produced on sterilized potato tubers are carmine red by species of section Discolor, Roseum, Sporotrichiella, and some of Gibbosum, and citric or sulphuric yellow in Neesiola subsection of Discolor. These colors are of the basic modification and consequently are not turned blue by the addition of alkali. The carmine red turns yellow with an addition of acid. No color contrast is observed in sections such as Eupionnotes, Arachnites, and Ventricosum. The stroma and aerial mycelium of these fungi have the color shades of their conidia. The color of sclerotia and sclerotial stroma is blue in some and brown in other groups, as discussed under sclerotia.

III. Minor characters

1. *Hyphae with swollen cells:* Not true chlamydospores (fig. 1 Y) as in *F. flocciferum* Cda. and *F. tricinctum* (Cda.) Sacc.
2. *Aerial mycelium:* Loose, dense, jellylike, cottony, radiate, zonate.
3. *Immersed mycelium*: Slimy or leathery sheet (stroma), that may or may not have a plectenchymic base. Section Eupionnotes.
4. *Coremialike aggregations of hyphae.* Aggregations of anastomosed hyphae as in Eupionnotes and Ventricosum. True coremia so far were not observed.
5. *Aromatic odor:* Somewhat similar to lilac odor, produced by a number of species of section Elegans such as *F. oxysporum* Schlecht., *F. hyperoxysporum* Wr., *F. zonatum* (Sherb. s. var.) Wr., *F. vasinfectum* Atk., and *F. cubense* Erw. F. Sm. The last three species have the strongest odor, especially when grown on rice.

IV. Abnormal characters

1. *Spore:* Exceptional size, irregularities in shape of conidia, swollen cells in germinating spores (fig. 1, W) and constricted cells in dry conidia (fig. 1, X). These abnormal characters, although frequently present, are not taken into consideration for taxonomic purposes and have been more fully discussed elsewhere (*1*).

2. *Mycelium:* With the exception of Eupionnotes, in which immersed growth of the mycelium predominates even under normal and favorable spore production, changes to an immersed gelatinous growth in general indicate degeneration and self-digestion accompanied by abnormal spore production or even sterility. This condition may be changed into the norm by transferring to various media or by selection of virulent aerial colonies from new agar plates. Fortunately the number of Fusaria showing a tendency to degenerate seems to be small. This tendency is notable in *Fusarium nivale* (Fr.) Sacc., *F. anthophilum* (A. Br.) Wr., *F. orthoceras* App. et Wr., and *F. flocciferum* Cda. The latter two fungi if inoculated into living potato tubers, thoroughly disinfected with formaldehyde, regain their normal behavior when reisolated and retransferred to sterilized media. In some cases, agar media too rich in sugar or too alkaline or acid in reaction favor degeneration. Conditions, however, that favor normal conidial production generally warrant longevity and sufficient constancy. On the same medium mycelium transfers often give only a sterile stroma, while a transfer of macroconidia increases the tendency to reproduce this stage. These facts if not fully understood may make the proper identification of certain species doubtful. They show the necessity of improving cultural methods.

Brown and Horne (*3*) in their studies of the genus Fusarium have given interesting details on the modifying effect of transfers from various parts of a given fungus. These authors have stated that the rate of spread of certain colonies fell off after a time and was reduced (staling), while other colonies developed into normal sporodochia or pionnotes with small and low septate or long and high septate conidia. Furthermore, "saltants" showing sectorial effects of some strains have been produced in Fusaria derived from six different isolations from apple. In the preliminary studies this wide range of variability was considered to be connected with one and the same fungus, *F. blackmani* Brown et Horne.

This variability of the mycelium and spore character has been found to occur with various fungi. Notable among these are two closely related Fusaria described as *F. anguioides* Sherb. and *F. anguioides* var. caudatum Sherb. (*5*). These two organisms are closely related to *F. anthophilum* (A. Br.) Wr. isolated from apple fruit in England. The abnormal variations found in these cases, though great, were not mentioned in the diagnosis of the Fusaria because normal characters were regarded as sufficient for determination.

PRODUCTION OF THE NORM

Cultural conditions which will produce good mycelial growth are not necessarily those most favorable for the production of normal spores. In the study of Fusarium it has been found necessary to use a variety of media for the production of the various normal mycelial and reproductive stages. The best media for these purposes at present available are the ordinary ones used in plant pathology investigations. A combination of a number of vegetable substances may prove valuable in some cases, but, so far, sufficient work has not been done to justify recommending such a medium. Because the same vegetable under different conditions varies in its chemical composition, it would be highly desirable to produce a satisfactory synthetic medium. It is hoped that a synthetic nutrient may sometime be found which will render it possible to control the production of spore bodies or sclerotia at will. Until some such new media are produced, vegetable media will be found to be fairly satisfactory for taxonomic purposes.

No one culture medium is at present used which will produce optimum development of all phases of fungus growth; hence certain media have been selected which proved most satisfactory for the best production of conidia, and other media for the production of sclerotia, mycelium, chlamydospores, and other characters. It must be understood, however, that not all species will react in exactly the same way, and therefore a medium which produces normal conidia in most species may need to be supplanted by another in the case of other species. The media used at the conference are given in Table I.

TABLE I.—*Culture media showing types of growth readily produced on each.*[a]

Media	Conidia	Chlamydospores	Sclerotia	Mycelium	Color
Potato tuber cylinders (no water added)	+	+	+	+	+(basic)
Oatmeal agar (Sherbakoff (5))	+		+		+(basic)
Potato agar+2 per cent dextrose (200 gm. to 1,000 c. c.)	+	+	+		
Potato agar+5 per cent dextrose (200 gm. to 1,000 c. c.)	+		+	+	+(basic)
Rice (2 gm. to 6 c. c. water)	+		+		+(acid)
Melilotus stems (mature stems+4 c. c. water)	+	+	+		Color of spores
Lupinus stems (mature stems+4 c. c. water)	+		+		Color of spores
Alnus (2 to 3 year twigs+4 c. c. water)	+		+		Color of spores

[a] All agar media contains 2 per cent agar. All media are autoclaved for 45 minutes at 10 pounds pressure except rice, which is steamed for one hour on three successive days. When necessary to use young Melilotus stems, 4 c. c. of a 0.5 per cent KOH solution should be used in place of water to neutralize the acidity of the stems.

In general, the use of several vegetable media, such as those mentioned in the table, will result in the production in good condition of the various characteristics of the fungus. In some cases special methods must be employed for the production of certain phases. The addition of acid to the standard potato agar greatly stimulates an abundant porduction of normal spores in such species as the Fusarium stage of *Gibberella saubinetii* (Mont.) Sacc. Substances poor in food value favor the production of chlamydospores. In species which produce chlamydospores only rarely, as *F. aurantiacium* (Lk.) Sacc., chlamydospore development may be brought about by growing in sterile tap water. The reaction of the medium is important from the standpoint of color production and, in some cases, greatly influences growth of mycelium and abundance of spore production.

In transferring, it must be borne in mind that the type of inoculum influences the resultant growth. Continuous transfer of one type of inoculum tends to production of that type of growth. Repeated transfer of mycelium or chlamydospores tends to good development of mycelium and sclerotia in those species which have sclerotia. For production of abundant spores, transfers should be made from sporodochia or pionnotes. When sporodochia are lacking and macrospores are not numerous, the chance of transferring mycelium may be eliminated by making dilutions in tubes of sterile water and transferring a loop full of the dilution to the culture medium, or by using the plate dilution method. The latter method affords an opportunity to select colonies showing the greatest tendency toward spore production.

Environmental factors other than the medium which should be taken into consideration are temperature, humidity, and light. There are indications, in some species at least, that temperature has an appreciable effect upon the morphology of the conidia as well as upon the rate of growth of mycelium (4). Perhaps to temperature may also be ascribed part of the effect on color intensity usually attributed to light. Humidity affects the nature of the culture, as is evidenced by the increased production of chlamydospores in some species and the production of swollen conidia in excessively moist media. The fact that these environmental factors have been given very little consideration in identification of Fusaria may be responsible for some of the difficulties encountered in various countries where taxonomic work is done.

IDENTIFICATION

If we realize the fact that tubercular sporodochia with normal and uniform conidia occur in the majority of Fusaria that can be easily grown in pure culture, there will be no difficulty in judging the normal stages and, consequently, in identifying most of these fungi. For the remaining forms more detailed studies will have to be conducted in order to produce or prove the absence of particular stages such as sporodochia, pionnotes, chlamydospores, and sclerotia. It should be stated also that even conidia normal in appearance often differ greatly in their size and shape according to where they are produced, whether on mycelium' over a wet surface or in a definite sporodochium. In a typical pionnotes long conidia may be produced, and in sporodochia they may be considerably shorter, as in *F. vasinfectum* Atk., *F. cubense* Erw. F. Sm., *F. lycopersici* (Sacc.) Wr., and others. Differences in size and shape may also appear when the fungus is grown on

various types of media. In section Roseum longer and slenderer conidia are produced on rice and oatmeal agar than on other media more favorable to sporodochial development. Because of these differences, it is necessary to pay particular attention to the average size of different types as grown upon different media and under different conditions. A comparison must be made only of conidia produced under comparable conditions and between comparable sporulation types.

Often the identification is difficult because certain species persistently produce only a microconidial stage. In such instances some special methods must be employed to induce the fungus to develop macroconidia. For instance, in the case of *F. chenopodinum* (Thum.) Sacc. only the comma-shaped spore (fig. I, D) may be observed for a long time. However, by repeated transfers of occasionally found macroconidia, finally normal macroconidia can be obtained in abundance. These conidia enable us to identify the fungus with ease. It is important to emphasize that, in this species as in some others, often an intermediate type of macroconidia is produced when the fungus is in a semimycelial stage of growth and when sporodochia are underdeveloped. These conidia in *F. chenopodinum* (Thum.) Sacc. can easily be taken for those of *F. sambucinum* Fuck. Generally the microconidia in themselves do not represent definite enough characters to be used for identification.

In those doubtful cases where normal macroconidia are not readily produced the presence of chlamydospores may be of extreme importance for determining the group. The presence or absence of chlamydospores makes it possible to separate species of section Elegans with terminal chlamydospores from section Lateritium with no chlamydospores, even though their macroconidia may be similar when grown under certain cultural conditions.

The presence of ascigerous stages in some of the sections is of additional help in the identification of the imperfect or conidial forms (*9*, *11*). A number of ascomycetes (Nectria, Calonectria, Hypomyces, Gibberella) have been grown from the ascospores in pure culture and developed the conidial stage, thus showing the relationship. In other cases cultures of Fusaria collected from nature have developed perithecia.

Color characters on various media rich in carbohydrates afford a fairly reliable means of placing the fungi into sections. The color of conidia and stroma especially represent an important complex for identification.

The ordinary procedure in determining Fusaria is to illustrate and measure normal spores and a few exceptions that show the variability and changes with increasing age. The standard magnification used for the drawings is 1–1000 for the spores and 1–500 for parts of sporodochia showing conidiophores and formation of spores. Smaller magnifications may be used for special purposes, such as groups of sporodochia, sclerotia, and stroma erumpent. For a short study water mounts are preferable. In order to prevent moisture from escaping during the procedure of drawing, the edges of the cover glass may be waxed. Another method (*7*) of preparing slides, which has the advantage of placing all the spores in one plane and holding them in place, is to make the mount on a very thin (0.5 mm.) sheet of agar placed on the slide. These agar sheets can readily be prepared by pouring clear agar between glass slides set the required distance apart. This method is very convenient for photographic purposes.

CLASSIFICATION OF THE FORM-GENUS FUSARIUM

The form-genus Fusarium includes all hyphomycetes and conidial stages of ascomycetes that have no black or pure gray color either in mycelium or in conidia and that have macroconidia that are acrogenous, typically septate, sickle-shaped, and not round at the ends. Microconidia, chlamydospores, and sclerotia may be present. Some Fusaria have been proved to be conidial stages of certain Hypocreaceae, such as Nectria, Hypomyces, Gibberella, and Calonectria. The fungi of the form-genus Cylindrocarpon, formerly considered as Fusaria, have Nectrias and certain Hypomyces (*8*) as perfect stages. Some of the species of Ramulara or similar fungi as Septomyxa also formerly placed under Fusarium, have as their perfect stage Neonectria (*10*, *11*) and Mycosphaerella (*11*). The fungi parasitic on scale insects and possessing sickle-shaped to fusoid conidia are referred to the genus Microcera, which is said to be the conidial stage of Sphaerostilbe.

GROUPING OF FUSARIA IN SECTIONS

Many Fusaria readily fall into separate groups possessing similar characters. These groups are called sections, which, on the basis of their apparent

relationship, especially when their perfect stages are considered, are arranged as follows: 1, Eupionnotes Wr.; 2, Arachnites Wr.; 3, Sporotrichiella Wr.; 4, Camptospora Wr.; 5, Arthrosporiella Sherb.; 6, Gibbosum Wr.; 7, Roseum Wr.; 8, Liseola n. n.[6]; 9, Lateritium Wr.; 10, Discolor Wr.; 11, Spicarioides N.comb.[7]; 12, Saubinetii Wr.; 13, Elegans Wr.; 14, Martiella Wr. (sensu extenos)[8]; and 15, Ventricosum Wr.

KEY TO THE SECTIONS OF FUSARIUM

With the exception of section Camptospora (*10*), which needs further study, all of the species are arranged in the following key to the sections:

a. Microconidia on aerial mycelium usually present and dominately 0-septate, ovoid, fusoid, reniform, or pearshaped.
  b. 0-septate conida pearshaped. Macroconidia when normal are in shape intermediate between those of the sections Elegans and Roseum, though more curved than either; substratum rose-colored, intercalary chlamydospores, or similar structures may be present. Sec. 3, *Sporotrichiella*.
  bb. 0-septate not pearshaped.
    c. 0-septate conidia in chains.
      d. Conidial walls thin. Microconidia mostly in chains, fusoid-ovoid; macroconidia in form and color similar to those of sec. Lateritium; no chlamydospores; substratum vinaceous-violet; some of the species are connected with Gibberellas of sec. Lisea (Sacc.) Wr.....Sec. 8, *Liseola* n. n.
      dd. Conidial walls thick or highly refractable. Macroconidia pluriseptate, in shape resembling those of the sec. Discolor.....Sec. 11, *Spicarioides* n. comb.
    cc. 0-septate conidia not in chains.
      d. Conidial walls thin. Macroconidia attenuate at the top ends, pedicellate; terminal and intercalary chlamydospores present; color of conidia brownish to salmon; no blue or green color in conidia even as a diffusion from stroma; stroma on artificial media principally vinaceous to lilac.....Sec. 13, *Elegans*.
      dd. Conidial walls relatively thick. Macroconidia somewhat truncate or rounded at the top end, or at least not distinctly attenuate, some times slightly constricted at the tip ends; terminal and intercalary chlamydospores present; color of conidia brown-white to golden brown with occurrence of green to green-blue as diffusion from stroma.....Sec. 14, *Martiella*.
aa. Microconidia on aerial mycelium usually absent or 0-3 or more septate, reniform, comma, spindle, to sickleshaped.
  b. Macroconidia apedicellate. Color type, orange to light salmon.
    c. Typical pionnotes always present; comparatively slow-growing fungi.....Sec. 1, *Eupionnotes*.
    cc. Typical pionnotes absent; comparatively fast-growing fungi.....Sec. 2, *Arachnites*.
  bb. Macroconidia subpedicellate to pedicellate.
    c. Terminal chlamydospores present; intercalary chlamydospores absent; no true sporodochia; macroconidia wedge-shaped to slightly sickle-shaped, not constricted at the top. Sec. 15, *Ventricosum*.

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[6] Liseola nn. (Syn. Constrictum Wr. pro parte subs. Elegantis; Moniliforme Sherb.*b*); Microconidiis plus minusve in catenulis, dispositis, fusoideis-ovoideis, macroconidiis forma et colore sectionis Lateritii, liberis, in sporodochiis, in pionnote; chlamydosporis nullis; stromaet violaceo. Status conidicus Gibberellarum sect. Liseae (Sacc.)

[7] Wr. Spicariodes (Wr. subsect.) n. comb. (Syn. subsect Spicarioides sectionis Discoloris Wr.) Microconidiis sporodochialibus pluriseptatis forma specierum sectionis Discoloris. Chlamydosporis nullis Stromate carmineo.

[8] *Martiella* Wr. (Sensu extenso sectionis Martiellam Wr. et Pseudomartiellam Wr. includendo). Macroconidiis dorsiventralibus fusoideofalcatis, apice rostrato truncato vel rotundato, basi plus minusve subpedicellata, in sporodochiis et pionnote sordide albo, ochroleuco vel aureo; stromate aerugineo fere nigrescenti, chlamydosporis terminalibus, intercalaribus, singulis, binis, catenulatis vel acervalibus. Status conidicus Hypomycetum sect. Pseudomartiellae.

cc. Terminal chlamydospores absent.
d. Intercalary chlamydospores present.
e. Sporodochia typically absent. Conidia, when free-borne on aerial mycelium, spindle-shaped. Macroconidia gradually attenuate, generally lanceolate, apedicellate; but also sickleshaped and pedicellate; color intermediate between that of Roseum and Gibbosum sections, sclerotia may be present....Sec. 5, *Arthrosporiella*.
ee. Sporodochia typically present.
f. Macroconidia with top ends much attenuated; stroma typically brown,[9] sometimes carmine[10]....Sec. 6, *Gibbosum*.
ff. Macroconidia with top ends somewhat truncate; conidia ochreous to salmon; stroma Roseum-like; blue sclerotia may be present....Sec. 10, *Discolor*.
dd. Intercalary chlamydospores absent.
e. Top ends of macroconidia gradually attenuate; when free-borne on aerial mycelium, sickle-shaped; or none. Acid-color modification of aerial mycelium yellow except in *F. anthophilum* (A. Br.) Wr., and other related fungi; conidial walls thin....Sec. 7, *Roseum*.
ee. Top ends of macroconidia somewhat constricted.
f. Conidial walls thin, and in this character, as well as in shape and color, similar to section Elegans....Sec. 9, *Lateritium*.
ff. Conidial walls thick, highly refracable, and in this character, as well as in shape and color, similar to section Discolor; conidial stage of *Gibberella saubinetii* and other similar Gibberellas....Sec. 12, *Saubinetii*.

RELATIONSHIP OF FUSARIA TO ASCOMYCETES

A number of different species of Fusaria have been definitely connected with certain ascomycetes of the Hypocreales group. In some other cases the connection is very probable, and in still other cases the connection is suggested as possible. For convenience the instances are mentioned here under different sections of the genus Fusarium.

Sec. 1. Eupionnotes. Perfect stage, *Nectria moschata* Glück, conidial stage similar to *F. aqueductuum* Lagh. var. *pusillum* Wr.

Sec. 2. Arachnites. Perfect stage, *Galonectria graminicola* (Berk. & Brome) Wr., conidial stage *F. nivale* (Fries) Ces.

Sec. 3. Sporotrichiella. No connection with an ascomycete is known.

Sec. 4. Camptospora. Perfect stage *Nectria episphaeria* (Tode) Fr., conidial stage may be *F. cavispermum* Cda.

Sec. 5. Arthrosporiella. No definite connection with an ascomycete is known.

Sec. 6. Gibbosum. No connection with an ascomycete is known.

Sec. 7. Roseum. *Gibberella tropicalis* Rehm. possibly has Roseum-like conidial stage.

Sec. 8. Liseola. *Gibberella acervalis* (Moug.) Wr., conidial stage very similar to *F. moniliforme* Sheld.

Sec. 9. Lateritium. *Gibberella baccata* (Wallr.) Sacc., conidial stage *F. lateritium* Nees; *G. pulicaris* (Fr.) Sacc., conidial stage *F. sarcochroum* (Desm.) Sacc.; *G. moricola* (Ces. et Not.) Sacc., conidial stage *F. urticearum* (Cda.) Sacc.; *G. effusa* Rehm, conidial stage *F. salicis* Fuck.; *G. evonymi* (Fuck.) Sacc., conidial stage *F. pyrochroum* (Desm.) Sacc.; *G. juniperi* (Desm.) Wr., conidial stage *F. fructigenum* Fr.

Sec. 10. Discolor. *Gibberella heterochroma* Wr., conidial stage *F. polymorphum*-like; and *G. cyanogena* (Desm.) Sacc., conidial stage *F. sambucinum* Fuck.

Sec. 11. Spicarioides. No connection with an ascomycete is known.

Sec. 12. Saubinetii. *Gibberella saubinetii* (Mont.) Sacc., conidial stage *F. graminearum* Schwabe; *G. flacca* (Wallr.) Sacc., conidial stage *F. caricis* Oud.

[9] Subsection Eugibbosum n. subsect.
[10] Subsect. Ferruginosum (Sect. Ferruginosum Sherb.) n. comb.

Sec. 13. Elegans. No connection with an ascomycete is known.

Sec. 14. Martiella. *Hypomyces ipomoeae* (Hals.) Wr., conidial stage *F. javanicum* Koord.; *Hypomyces cancri* Wr., conidial stage *F. striatum*-like; *Hypomyces leptosphaeriae* (Niessl) Wr., conidial stage *F. sphaeriae* Fuck.

Sec. 15. Ventricosum. *Hypomyces solani* Rke. et Berth., conidial stage *F. argillaceum* (Fr.) Sacc.

NOMENCLATURE IN RELATION TO SPECIES, VARIETIES, AND FORMS

The plan recently considered and approved by the committees of the Phytopathological Society, the Society of Agronomy, and the Mycological section of the Botanical Society on June 6, 1924, in Washington, D. C., is followed in regard to questions of nomenclature and terminology. This plan provides for the use of the Latin trinomial composed of the genus, species, and variety. The *species* includes groups of individuals which can be separated on the basis of morphological character of such a nature as to be applicable and usable by mycologists in general and which will be most serviceable for practical purposes. The *variety* is distinguished by morphological characters, but less important than those used for specific segregation. An additional category termed *forma* is to be applied to subdivisions of the species or varieties characterized and distinguished primarily by physiological instead of morphological characters, though in some instances there may be present also some slight morphological, distinguishable differences. The *forma* is designated by an arabic numeral.

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THE HAM BEETLE, NECROBIA RUFIPES DE GEER[1]

By PEREZ SIMMONS, *Associate Entomologist*, and GEORGE W. ELLINGTON, *Junior Entomologist, Stored Product Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

The principal species of insects and mites which damage smoked meats fall into two rather distinct groups: (1) Those that infest newly smoked, juicy meats—the cheese skipper (*Piophila casei* L.) and several species of blowflies (*Lucilia sericata* Meig., etc.,); (2) those that infest meats which have become dried to some extent by evaporation during long storage or as a result of prolonged smoking, or both—the ham beetle (*Necrobia rufipes* De G.), the larder beetle (*Dermestes lardarius* L.), the leather beetle (*Dermestes vulpinus* Fab.), and certain mites. The species described in this paper is the most important of the second group, and it is sporadically very injurious where smoked meats are stored for rather long periods. A large part of the expense of protecting cured meats with wrappings, sacks, and washes may properly be charged to this insect.

Riley (*24*)[2], who made the first economic investigation of the insect 50 years ago, cited cases of extensive injury to hams in St. Louis and Boston. In the dispute arising because of the infested stocks at Boston the consignee claimed that the husk paper in which the consignor had wrapped the meat was likely to generate the worm. The referees of the case deposed, however, that "a warm, damp atmosphere and want of free circulation of air on the hams will produce or generate the worm in light-salted sugar-cured hams."

During the summer of 1921 a severe infestation developed in dry-cured Army bacon stored in crates at Baltimore and later at Washington. There were about 220,000 pounds of this bacon, and it was reconditioned by extensive trimming, which in many cases reduced the weight of sides by 75 per cent.

Howard (*16, p. 105–107*) defined the status of the pest as it is at present when he stated, in 1902, that it is hardly a species which causes a constant drain on the trade, but occasionally becomes extremely abundant, ruining large quantities of cured meats.

SYSTEMATIC POSITION, SYNONYMY

This species is the most injurious of the coleopterous family Cleridae, the larvae of which are typically predacious and often beneficial as enemies of economic insects, including the tobacco beetle (*Lasioderma serricorne* Fab.) and many species which attack forest trees.

De Geer (*12, p. 165*) published the original description as *Clerus rufipes* in 1775. In 1796 Latreille (*18, p. 35*) erected the genus Necrobia. Mulsant and Rey (*22, p. 122–124*), who placed the species in the genus Agonolia, listed *Clerus rufipes* De Geer, Oliv., *Dermestes rufipes* Fab., *Corynetes rufipes* Herbst., etc., *Necrobia rufipes* Oliv., etc., as indicating the four genera to which the present species had been referred:

The following specific references (*27, p. 142–143*) are synonymous with *rufipes:*

amethystina Steph., 1832, Ill. Brit. Ent. 5: 417; Klug, 1842, Clerii, Phys. Abh. K. Akad. Wiss. Berlin for 1840, p. 394.
dermestoides Pill. et Mitterp., 1783, It. Poseg., p. 68, pl. 7, fig. 8.
foveicollis Schkig., 1900, Mitt. Nat. Mus. Hamburg 17: 20.
glabra Champollion, 1814, Millin Mag. Encycl. 3: 44; 1902, Schenkling, Bul. Mus. d'Hist. Nat. 8:332.
mumiarum Hope, 1834, Pettigrew, Hist. Egypt. Mum., p. 54, pl. 5, figs. 1–3; Schenkling, op. cit., p. 332.
pilifera Reitt., 1894, Verh. Nat. Ver. Brünn 32: 85; Abeille, 1895, Bul. Soc. Ent. France 1: 208.

COMMON NAMES

The common name "red-legged ham beetle" was given to the insect by Riley (*24*) in 1874. An article in the Yearbook of the United States Department of Agriculture for 1907 (*1, p. 552*) referred to the pest as the "ham beetle." Dealers in meats know the insect by the name "paper worm."

[1] Received for publication April 22, 1924; issued June, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 863.

According to Froggatt (*10, p. 26*) *N. rufipes* is known in the Pacific Islands as the "copra bug."

Inasmuch as the other beetles which sometimes attack smoked pork already have well-established common names—"larder beetle" and "leather beetle"—the writers prefer the name "ham beetle" for *Necrobia rufipes* De Geer.

THE ADULT

TECHNICAL DESCRIPTION

Form oval, sides subparallel, widest at apical fourth. Color blue, sometimes with violaceous or greenish luster, legs and first five segments of antennae castaneous, terminal six segments of antennae and the trophi piceous, eyes black, venter aeneous black. Head rather sparsely punctured, the punctures large and small intermingled, median portion of frons and vertex with very few punctures. Pubescence sparse, erect, and black. Eyes finely granulated, with a triangular emargination approximate to the antennal insertion. Labrum emarginate. Both maxillary and labial palpi with subcylindrical, slightly acuminate terminal segments. Antennae each with eleven segments; the first thick, slightly bent; the second about one-third the length of first, equilateral; third almost twice the length of second but of equal thickness; fourth to eighth mutually equal in length, each just perceptibly wider than the one preceding; the eighth is transverse; ninth and tenth strongly transverse, subequal, about one-half as long as broad; the eleventh almost square. Pronotum transverse, sides evenly curved from base to apex, basal and apical angles very obtuse, almost wanting, lateral cariniform margin distinct, finely serrulate. Surface rather sparsely set with moderately coarse punctures; punctures much more dense at sides than on disc; pubescence as on head. Scutellum small, transverse. Elytra long, suture closed, lateral margin finely beaded, each with nine distinct rows of punctures, the normally occurring tenth row being confused with the ninth; rows obsolete just behind the middle, surface between puncture rows and of apical portion rather densely set with fine punctures from each of which a posteriorly-directed subrecumbent black hair arises. Under parts and legs rather finely and densely punctured, clothed with pale fulvous pubescence with a few longer black hairs interspersed. Legs moderately long, femora not greatly enlarged, tibiae straight, tarsi short, of five segments of which the fourth is very small and concealed between the lobes of the third, first three segments with lamelliform pads beneath. Claws rather long, provided at base with a broad toothlike appendage. Length: 3.5 to 7 mm.[3]

In the female each of the elytral punctures, which are arranged in rows, gives rise to a stiff black hair slightly inclined anteriorly; in the male these hairs are subrecumbent and directed posteriorly. Other secondary sexual characters are absent.

ADULT BEHAVIOR

After the transforming insect becomes adult, it gnaws an irregular hole in the wall of the pupal cell, and emergence occurs. The meconium is voided in the cocoon. Sometimes a day or two elapse between the time the adult becomes fully pigmented and its escape, and in the case of adults emerging in vials they often return to the cell for concealment.

Mating usually occurs promptly after two newly emerged beetles of opposite sex are placed together, and is frequently observed during the long oviposition period, especially when the beetles appear frightened during manipulation of the dishes in which they are confined. The forwardly directed elytral spines of the females doubtless materially assist the males, which are usually smaller than the females, in maintaining their position during copulation.

Besides sharing in the attacks of the larvae upon ham and cheese, the adults are markedly predacious and also cannibalistic. It is apparent that the beetles and their larvae can destroy an infestation of cheese skippers (*Piophila casei* L.) in ham,[4] and under some conditions, as in stores of bones, they probably are beneficial to the extent that they help destroy the maggots of skippers and blowflies. In the laboratory experiments fat bacon was a much less favored food for adults than skipper larvae.

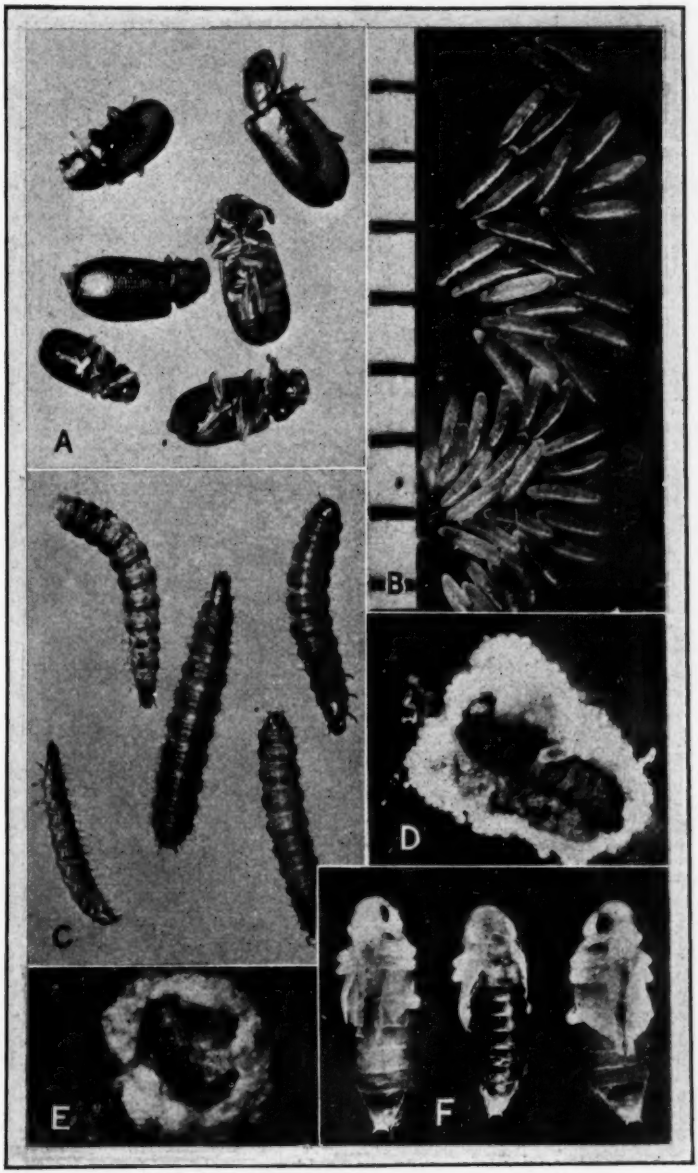
Even when fed daily with skipper maggots, ham beetles sometimes dismember an individual of their own species and then proceed to devour it. Specimens which die naturally are usually promptly eaten. The beetles have also been observed by the writers to eat the eggs and larvae of their own species, and where adults become very numerous, as has occurred in boxes of smoked meat under observation, their cannibalism is apparently responsible for a great reduction in the number of their larvae. Adults which are deprived of food usually die in two or three weeks.

During hot weather the beetles may be seen in slow flight about infested rooms. The usual mode of progression, however, is by rapid running. When roughly handled they feign death for a short time; as a rule they are negatively phototropic and quite wild, but those which are confined and exposed to light during the day and fed frequently become increasingly tame. On being held in the fingers or forceps a strong, very disagreeable, but transient, odor is emitted.

A photograph of several ham beetles is shown in Plate 1, A.

[3] The writers are indebted to Edward A. Chapin for the foregoing technical description.

[4] A similar service in stored products by a clerid has been reported by G. A. Runner (*26, p. 35*), who stated that *Thaneroclerus girodi* Chevrolat apparently at times causes the complete disappearance of *Lasioderma serricorne* Fab. from boxes of infested cigars.



Necrobia rufipes

A.—Adults. ×4
B.—Eggs laid on cardboard. (Showing the edge of a millimeter scale)
C.—Full-grown larvae. ×3
D.—Larva in cell made between plug of black cotton and side of vial
E.—Pupal cell on canvas sack, opened to show pupa and cast prepupal skin
F.—Pupae

THE EGG

The egg of *Necrobia rufipes* is about 1 mm. in length and 0.25 mm. wide, tapered, and roundly pointed at both ends and slightly curved in outline. It is smooth, shining, translucent, and is glued in place. As shown in Plate 1, B, the eggs are usually deposited in clusters. Those laid by old females often partially collapse laterally within a few hours after being deposited, and such shrunken eggs do not hatch.

Toward the end of the incubation period (four or five days in length during warm weather) the four eyespots of the embryo become visible, followed by pigmentation of the tips of the mandibles. Shortly after hatching, and in some cases before the escape of the larva, the caudal plate, head capsule, and prothoracic shield assume their final color.

HATCHING

The struggles of the hatching larva cause the posterior extremity to move about, with the result that the tubercles on the caudal plate tear the eggshell open at one end, the mandibles accomplishing the same at the anterior end. Thus the eggshell is usually torn open at both ends before the larva leaves it, and the larva often remains in the shell as in a short tunnel for several hours, feeding on the shell.

THE LARVA

TECHNICAL DESCRIPTION (*4, p. 597–599*)

Total length of body, about 10 mm.; extreme width, about 2 mm.; fifth to seventh abdominal segments widest; anterior width of prothorax one-half the width of the seventh abdominal segment; extreme thickness, 1½ mm.; seventh abdominal segment thickest. Corneous parts shiny, brown ocher; delicately chitinized parts shiny, pale clay yellow; membranous parts of thorax and abdomen dorsally mauve or lilac with white muscle attachments, ventrally whitish with bluish pattern. Frons rugose, anteriorly on each side of middle line with a shallow deepening. Labrum three times as wide as long; width about one-third the length of frons. Mandibles half as long as frons; length to width as 4 : 2; retinaculum and tooth same size, well developed, and rather obtuse. Two short mandibular setae. Prothoracic shield two-thirds as long as wide, with parallel sides. Both mesothorax and metathorax are about as long as prothorax, surpassing it one-third or more in width; metathorax a trifle wider than mesothorax; mesothoracic and metathoracic dorsal plates present, small, and about the same size. Basal plate of cerci a trifle wider than the prothoracic shield, length to width as 2 : 3. Cerci one-third the length of basal plate, upward curved, diverging about 60°.

LARVAL BEHAVIOR

The delicate, wrinkled, hairy larva after leaving the shell moves about but little for awhile, confining its activities as it gains strength to feeding on the unhatched eggs in the near vicinity and eating the shells of empty eggs. The shells are usually almost wholly consumed. New-laid eggs of the ham beetle, exposed to very young larvae which were also provided with dead skipper maggots, were all eaten. Eggs of *Dermestes vulpinus* Fab. were also eaten.

The postembryonic larvae are repelled by light, and, for the first day or so, prefer to spend most of their time hidden beneath some object, even when food is provided and light excluded.

The rearing of individual larvae (Table VII) was found to be practically impossible when fat bacon or skipper larvae were used as the only food. By feeding the postembryonic larvae with eggs of the ham beetle for 8 or 10 days after hatching, then giving both eggs and crushed skippers for 4 days and crushed skippers thereafter, single larvae were easily reared. To prevent the very small larvae from escaping or becoming entangled in cotton, the best container was found to be No. 11 veterinary capsules, the food being placed between two pieces of cardboard. The larvae thus reared reached a good size and molted two or three times before pupating. First skins measured 0.08 mm. between the tips of the caudal tubercles, second skins 0.25 mm., and third skins 0.5 mm. The molting skin splits over the thorax, the head is withdrawn from the head capsule and extruded through this opening, and the insect crawls out of the skin. There is much variation between the dimensions of the smallest and largest full-grown larvae, the latter being 100 per cent larger than the former.

Larvae of all instars are repelled by light. Well-grown larvae are able to crawl rapidly and to kill migrant skipper larvae. In their efforts to escape the maggots throw their attackers about with considerable force, but the latter seldom release their jaws until the maggots have become helpless. When touched, ham-beetle larvae protect themselves by thrashing about, bending their extremities together first on one side and then on the other. The full-grown larva is shown in Plate 1, C.

PUPAL CELL OR COCOON

Following the completion of feeding, full-grown larvae infesting smoked meat migrate from the greasy material in which they develop and seek a dark, dry spot in which to build the cocoon. At this time the larvae will not usually eat if skippers are offered to them.

The cocoon (pl. 1, D, E; pl. 2, D) may be completed within 24 hours, and is formed by filling in the open boundaries of the crevice chosen for pupation with a wall of white substance which is vomited at will from the mouth of the larva in frothy droplets. Each droplet appears only after the larva has chosen the location for the next unit of the wall, and it hardens into a vesicular mass immediately it is put in place.

During the process of cell building the larva is usually curled in the cell, although sometimes the inclosure is large enough to allow it to extend its length. At times cocoons are broken into by adults and the occupants devoured. It sometimes happens that two larvae inclose themselves in a common cell. On one occasion a larva was observed to cease construction work and to remain motionless, apparently as a means of protection, for several minutes while another larva was crawling on the outside of the cocoon. Frequently, when collecting immature material for experimental use, the writers secured numbers of larvae by opening cells, and many of these were able to resume normal activity promptly and to secrete without further feeding sufficient froth to construct another complete pupal chamber.

HE PREPUPA AND PUPA

Several days after the cocoon is completed the larva contracts in length, the body consequently becoming more robust; the head assumes a fixed attitude at right angles to the body axis, and the insect becomes a prepupa. The last larval skin is next cast and the pupa appears in the cell.

The pupa is restricted in movement to wriggling of the abdomen, to the tip of which the shriveled cast skin of the larva usually adheres. Unprotected pupae are readily devoured by adults. Pupation occasionally takes place without the protection of a cell. The pupa is illustrated in Plate 1, F.

DISTRIBUTION

In 1804, Latreille (*19, p. 156*) gave the distribution of the species as southern France and Italy. Stephens in 1830 (*32, p. 327–328*) reported it rare about London, though rather abundant in certain years. According to Curtis (*6, pl. 350*) the range of the insect was extensive, including southern France and Africa, although in Britain it was rare. Sharp (*30, p. 254*) stated that it is one of our most cosmopolitan species. Houlbert and Bétis (*15, p. 16*) gave its distribution in Brittany as widespread, but quite rare.

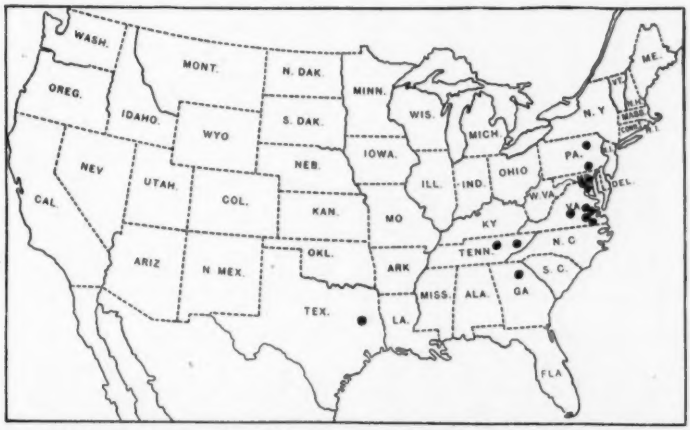


FIG. 1.—Map showing localities from which complaints of damage by the ham beetle (*Necrobia rufipes*) to smoked meats have been received by the Bureau of Entomology. Records of infested cargoes landed at various ports are not included

According to Lintner (*20*), the insect was introduced into the United States, and Froggatt (*9, p. 169*) believed the same to be true with respect to Australia.

Reports of injury to smoked meats have been received by the Bureau of Entomology from Texas, Tennessee, Georgia, Virginia, the District of Columbia, Maryland, and Pennsylvania, as shown by Figure 1.

In general, it may be said that *Necrobia rufipes* is a cosmopolitan insect. It is a species commonly

brought into our ports in cargoes. Records in the Bureau of Entomology show that it has arrived in cargoes in fish "guano" and bone meal from Honolulu, in garlic from New Zealand (probably having developed in other material), in coconuts from Manila, in bones from Argentina, in copra from the Philippines, in palm-nut kernels from British West Africa and Liberia, in herring and whale "guano" from Scandinavia, in dried egg yolk from China, in coconut palm from Ceylon, and in rattan from Japan. In bone storages it is frequently found in company with a closely related but economically unimportant species, *Necrobia ruficollis* Fab.

In the United States, as shown in Figure 1, reports indicate that most of the injury occurs in the Middle Atlantic States and that it is particularly marked in Virginia. Possibly this is due in a measure to the long storage of stocks of "Virginia" hams, which are not considered prime until they are about a year old.

SUBSTANCES INJURED

The first record of injury to human food by *Necrobia rufipes* seems to be that of Glover (*13, p. 97–98*), who reported it on cheese in Maryland. Riley (*24*) gave the earliest account of extensive injury to cured meats, concluding that attacks occur particularly to hams injured by overheating or by exposure to sun and rain. The species, he believed, is attracted by the fatty slime on hams.

Our chief concern in this country is with the infestation of smoked pork, other materials being attacked only on rare occasions.

The following list summarizes the known foods of this insect. The "substances infested but not fed upon" are sought by the migrating, full-fed larvae as suitable materials in which to pupate. Baled cotton and wool are sometimes badly matted with the pupal cells of the insect when these goods are carried in the same ship with bones.

Foods of the larvae or adults of the ham beetle, as recorded from literature, include: Cheese (*25, p. 226; 31, p. 266*), hams, bones (*34, p. 161; 31, p. 266*), fish (*25, p. 226; 31, p. 266*), drying carrion (*31, p. 266*), copra (*10, p. 26; 2*), hides (*5*), salt fish (*7*), bacon, dried egg (*29*), dried figs (*17*), and Egyptian mummies.[5] Bureau of Entomology records include: Dried egg yolk, hams, cheese, fish "guano"[6], bone meal, bacon, copra, bones, palm-nut kernels, and herring and whale "guano."[6] Substances infested but not fed upon, as recorded in published accounts, include: Silk (*28, p. 426*), baled cotton (*21*), and woolen tops (*11*); rattan and salt are given in the files of the bureau. A number of other references to literature, for the most part dealing with infestations of copra, are not included.

NATURE OF INJURY

Both larvae and adults feed upon smoked meats, the latter superficially. The larvae at first burrow beneath the hide, later extending their feeding deeper into the meat, chiefly in the fat portions (pl. 2, C). Frass is extruded from the burrows; this is shown in Plate 2, F. A piece of meat which had been seriously damaged on the flesh side is illustrated in Plate 2, A, and the work of the larvae in perforating a grease-soaked paper wrapping is shown in Plate 2, E. Plate 2, B shows the work of this species in old cheese.

BIOLOGICAL INFORMATION RECORDED BY OTHER WRITERS

The life history and habits of *Necrobia rufipes* have received little attention. Riley (*24*) stated that hibernation takes place solely as the larva and that no adults emerge before the first of May. On the other hand, Fay (*8, p. 197*) listed it among insects secured in winter collections.

[5] See reference to Hope on *Necrobia mumiarum*, under "Synonymy."
[6] Refuse used as fertilizer.

EXPLANATORY LEGEND FOR PLATE 2

Necrobia rufipes

A.—Longitudinal section of an old infested ham. In this case most of the feeding has been on the inside and has extended toward the skin side. Burrows may be seen penetrating the solid fat. Extensive feeding took place under the skin at the shank end, some of which is shown in the photograph

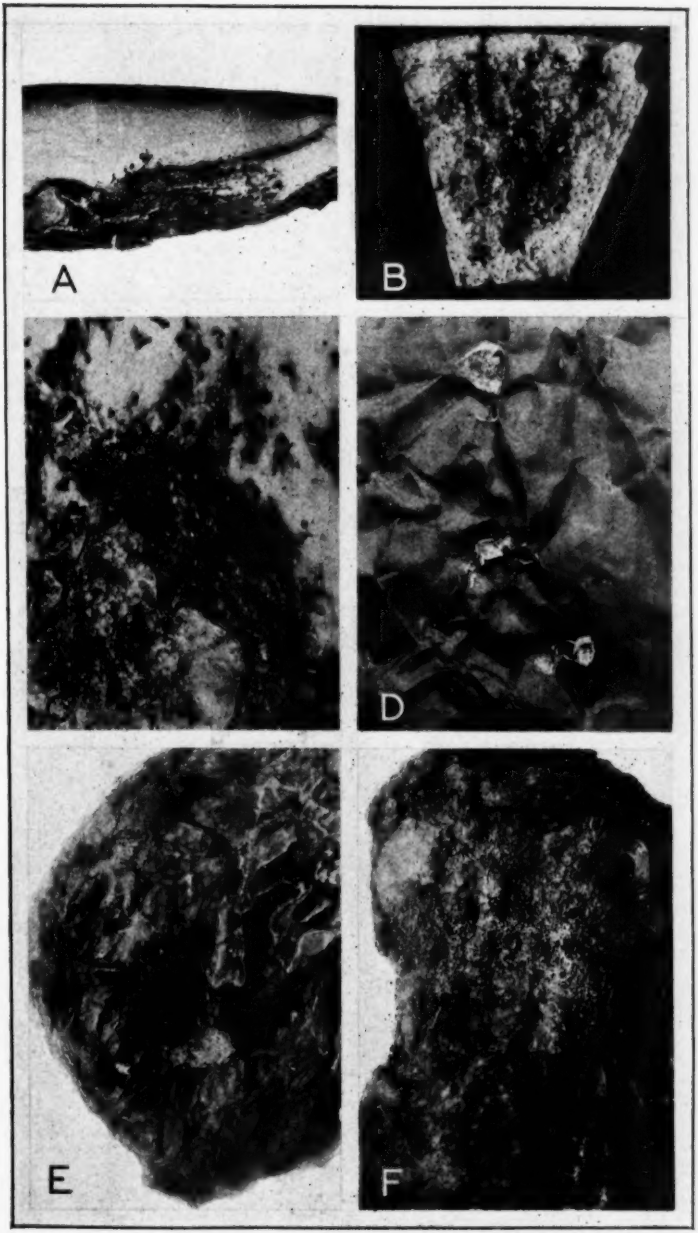
B.—Injured cheese. Several pupal cells are present in the excavated area. The dark spots are the excrement of the adult beetles. In this case the beetles and their larvae superseded an infestation of the cheese skipper (*Piophila casei*)

C.—Feeding burrows of larvae in the fat of an old ham. The cut surface, to the right of the black ink line, slants down into the tissues

D.—Pupal cells formed in creases of paper wrapper on ham. Cells torn open when paper was flattened out

E.—An old ham which had been wrapped for several months. The inner paper had become grease-soaked and the larvae penetrated it in the course of their feeding

F.—An old shoulder infested with larvae, showing the mealy frass from their burrows which has accumulated on the surface



(For explanatory legend see p. 850)

The beetles appear in May and June, according to Howard (*16, p. 105–107*), and never seem to oviposit except where the meat is more or less exposed. He also stated that E. A. Schwarz found adults in midwinter at Detroit, Mich., and Cambridge, Mass.

Herrick (*14, p. 278*) reported that pupation takes place in the more fibrous parts of ham, or sometimes in a near-by beam. The globules of white substance which form the cocoons are emitted from the mouth of the larva.

Of the biology of the ham beetle as a copra pest, Dupont (*7*) wrote that "it is supposed to be a purely predacious insect, feeding on dipterous and microlepidopterous larvae," but it was not known whether or not it attacks the larvae of *Oryzaephilus surinamensis* L. (the saw-toothed grain beetle), which are also abundant in copra stores.

LIFE-HISTORY INFORMATION

OVIPOSITION

The act of oviposition has never been observed by the writers; presumably the eggs are deposited when the insects are in darkness. Owing to their carnivorous appetites the beetles devour eggs which are exposed, and the females usually deposit them in crevices inaccessible to the mandibles of roving adults.

Following unsatisfactory oviposition trials with pieces of stale bacon attached to strips of cardboard, Petri dishes were used in which the only suitable place for oviposition was between small squares of dark-colored cardboard held together with a paper clip. In dishes which were greasy throughout, oviposition was sparse until clean dishes and dry cardboard were provided. It

TABLE I.—*Incubation period of Necrobia rufipes, 1922*

Date eggs were laid [a]	Hatching began	Minimum incubation period	Temperatures during each period [b]			Date eggs were laid [a]	Hatching began	Minimum incubation period	Temperatures during each period [b]		
			Max.	Av.	Min.				Max.	Av.	Min.
		Days	°*F.*	°*F.*	°*F.*			*Days*	°*F.*	°*F.*	°*F.*
Mar. 28	Apr. 5	8	79	66	53	Sept. 24	Oct. 2	8	75	68	60
May 9	May 15	6	82	74	70	25	Oct. 3	8	75	68	60
11	May 16	5	82	75	70	26	Oct. 4	8	74	68	60
12	May 17	5	77	74	69	27	Oct. 5	8	77	69	60
15	July 19	4	82	77	69	28	...do....	7	77	70	66
20	July 24	4	84	79	72	29	Oct. 6	7	79	70	65
21	July 25	4	89	81	72	30	...do....	6	79	71	65
25	July 29	4	84	78	71	Oct. 1	Oct. 7	6	79	72	65
Aug. 16	Aug. 20	4	88	81	73	2	...do....	5	79	72	65
19	Aug. 25	6	83	75	66	3	Oct. 8	5	79	73	67
20	...do....	5	80	74	66	6	Oct. 11	5	78	73	67
21	Aug. 26	5	80	74	66	9	Oct. 17	8	77	68	59
23	Aug. 28	5	81	77	68	10	Oct. 18	8	77	68	59
24	Aug. 29	5	81	76	69	11	Oct. 20	9	77	67	59
25	Aug. 30	5	81	76	69	12	Oct. 21	9	77	67	59
26	Aug. 31	5	81	75	69	13	...do....	8	77	67	59
27	Sept. 1	5	80	75	69	14	Oct. 23	9	77	67	58
28	Sept. 2	5	80	74	68	15	Oct. 24	9	75	67	58
29	Sept. 4	6	80	75	68	16	Oct. 26	10	77	67	58
30	...do....	5	80	75	68	17	Oct. 27	10	77	67	58
31	Sept. 5	5	82	76	68	18	Oct. 28	10	77	67	58
Sept. 1	...do....	4	82	76	68	19	Oct. 30	11	77	66	58
2	Sept. 6	4	82	77	72	20	Oct. 31	11	77	65	56
3	Sept. 7	4	84	78	73	25	Nov. 5	11	77	66	56
4	Sept. 8	4	85	79	75	26	...do....	10	77	66	56
5	Sept. 9	4	85	79	75	27	Nov. 7	11	77	66	56
6	Sept. 10	4	85	78	75	28	...do....	10	77	66	56
7	Sept. 11	4	85	78	75	Nov. 3	Nov. 14	11	77	67	58
8	Sept. 12	4	83	78	75	4	...do....	10	77	67	58
9	Sept. 13	4	83	78	75	6	Nov. 16	10	77	67	58
10	Sept. 14	4	83	76	70	7	Nov. 17	10	77	67	58
11	Sept. 15	4	83	76	70	8	Nov. 18	10	77	67	58
12	Sept. 16	4	81	75	70	12	Nov. 22	10	76	66	56
13	Sept. 19	6	81	72	58	16	Nov. 29	13	77	64	49
14	Sept. 20	6	81	71	58	17	Dec. 2	15	79	64	49
15	Sept. 21	6	81	70	58	24	Dec. 7	13	79	64	49
16	Sept. 23	7	80	70	58	28	Dec. 9	11	79	65	53
20	Sept. 28	8	76	68	60	29	Dec. 13	14	79	66	53
21	Sept. 29	8	76	68	60	Dec. 1	Dec. 14	13	79	66	53
22	Sept. 30	8	76	68	60	2	...do....	12	79	66	53
23	Oct. 2	9	76	69	60						

[a] Number of eggs laid each day varied from about 25 to 100.
[b] Average temperatures computed from thermograph readings every 2 hours.

is concluded that a close, dry crevice is preferred by the laying females.

INCUBATION PERIOD

At temperatures of 70° to 85° F. the usual incubation period is four or five days. In early winter, when temperatures became low in the laboratory at night, incubation periods of two weeks were recorded. Batches of eggs hatched with uniformity as regards time; the last eggs to hatch produced larvae about one day after hatching began, in warm weather. Table I shows incubation periods observed in 1922 at Washington, D. C.

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The results of oviposition in vials containing stale bacon as food for the adults show an average of 137 eggs and a maximum of 312, which does not indicate that the ham beetle is unusually prolific. When the beetles are fed on maggots, however (see Table IV), a far greater capacity for increase is indicated, and it is instructive to compare Table IV with Table II.

The eggs laid by females fed with maggots of the skipper fly in several cases totaled over 1,000 and were obtained in the following manner. Pairs were mated shortly after emergence from the cocoon and were fed daily, except Sunday, with three migrant larvae of *Piophila casei*, maggots in excess of three being usually uneaten. The results of the oviposition of some of the pairs are given in Table III, which includes 20 of the best records from a series of 76 pairs. The discontinuance of another series of 32 pairs, mated in March, 1923, became necessary after six of the females had laid from 444 to 1,110 eggs each.

TABLE II.—*Oviposition and longevity of Necrobia rufipes, 1922*

[Food: Stale fat bacon]

Pair No.*	Total eggs laid	Male emerged	Male longevity	Female emerged	Female longevity	Pair mated	Mating to oviposition	Duration of oviposition	End of oviposition to death of female	Laboratory temperatures (° F.)			
										Month	Maximum daily mean	Average daily mean	Minimum daily mean
			Days		*Days*		*Days*	*Days*	*Days*				
1	312	Feb. 23	135	Feb. 23	.....	Feb. 27	.....	.....	.....	Feb.	71	67	59
2	10	Mar. 4	.....	Mar. 4	49	Mar. 6	24	6	17	Mar.	72	67	59
3	155	Mar. 5	.....	do	.....	do	.....	.....	.....	Apr.	80	69	58
4	140	Mar. 6	186	Mar. 3	169	Mar. 8	.....	.....	.....	May	79	73	67
5	78	Mar. 7	160	Mar. 7	160	do	22	107	30	June	86	79	70
6	239	Mar. 9	104	Mar. 9	142	Mar. 10	14	97	30	July	87	79	71
7	68	do	158	do	175	do	.....	.....	.....	Aug.	83	76	71
8	109	do	163	do	183	do	26	104	52	Sept.	81	73	62
9	38	Mar. 10	85	Mar. 10	90	do	14	35	41				
10	111	Mar. 11	118	Mar. 11	161	Mar. 14	16	111	31				
11	233	do	.....	do	94	do	6	85	0				
12	192	Mar. 13	87	Mar. 10	137	do	6	72	55				
13	94	Mar. 15	.....	Mar. 15	.....	Mar. 18	24	88	.....				
Total	1,779	.....	1,196	.....	1,360	.....	152	705	256				
Average	137	.....	133	.....	136	.....	17	78	32				

* These pairs were used in preliminary experiments, but the results clearly show that an exclusive diet of smoked pork is not the most favorable food for adults kept in close confinement. Compare with Table IV.

TABLE III.—*Oviposition of Necrobia rufipes*

[Food: Larvae of *Piophila casei* L. Legend: EM, emerged and mated]

Date of oviposition	Eggs laid by female of pair No.																				Daily mean temperature
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1922																					°F.
Sept. 1	EM																				73
6		EM	EM	EM																	80
8	11	18		10																	76
9		31	6	37																	77
10		20	54	25																	78
11		3	38	34	EM																79
12		10	7	32		EM															77
13			36																		73
14			10	14																	74
15	1		16	15																	77
16		13	8	25			EM	EM	EM	EM											76
17			25			17															71
18						12					EM	EM									65
19													EM	EM							68
20		10	17				5								EM						69
21				6												EM					71
22			16	13							22										69
23		26					6				6										70
24		22	21	24		29	3	6			33		12								73
25		44	25			3	6				15		24								67
26						12															62
27							6	1					3								65
28	12	32				31	22				12		19								68
29		8																			70
30		20	17			8	16				5										70
Oct. 1			17								14										69
2	8					22															69
3		16	8			10					14					5					70
4			26			18					11		12								73
5	7	21	9	6		14	5				28										74
6		21	21			3	15	12			13			5			EM				74
7		19	8	20		23	19			14	16			13		3					73
8	6	27	12					10		8	25	10				11					74
9	3	17	23			21				2	8					9					71
10	5	19				20	2	18						6							73
11	2		13	2		16	9			2	16		5	6		16					67
12		33	10				12				14			11							64
13						4															65
14			12	9		19	7	14			6		29	9							68
15				5							23					27					68
16		19	28			31					20							EM			69
17		16	5							2	9			3							70
18		10	11											17				13			68
19		17											17					28			67
20			13			22								13	5	19		20	EM		66
21											10							17		EM	65
22						17															60
23				5		15	3				6			4		7		18			65
24		44	14				6				8							8			68
25			8			3				8		4	23	19			8	13			66
26			4			3					27			6				24			67
27																		6			65
28	7		23			25						12				7	34	3	9		67
29													6	5							62
30	5					8															65
31	8																	19			64
Nov. 1			8			4							14	5		8			9		68
2	9	11						8			8	5		8		8		7	7		68
3	5		21							9	11			5	6	16		23			68
4			18						13		17			16	14				8		68
5			4									20	9			10		13	15		69
6	3	20	11			31					12					3		9			68
7	8		9											14							69
8			23			28			1	31				21				28			66
9			26			24			14					10	5			26	9		67
10	9		15			16			5	14	18		10	16	11	20		10	14		66
11		24	24	10					6		5		17	12				6			67
12			8			30				9											62
13	23		28								6	8						15	11		67
14			9			17				14	18		25	18		8		16	24		67
15						17					6					3					69
16	13		28	4						6	8			13				5			67
17	12		3			33				10	8			6				21			67
18	16		21				7				13			13					10		67
19	6		18			27								9					7		63
20						12															64
21	7					12										14		55			65
22	14					10					7		10	14	5						63
23	9												11								64

TABLE III.—*Oviposition of Necrobia rufipes*—Continued

Date of oviposition	Eggs laid by female of pair No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Daily mean temperature
																					°F.
24			9								13										66
25											7								9		66
27			18											14							63
28	10												21						14		65
29			26							13						9					67
Dec. 1										17	4								6		66
2			27							9	11										68
5	10		38								5			(a)				26			69
6													4			4					65
7	19		12			5				5											65
8			22							5		5									67
9	10																				71
11	19					23				11	6	12	16					13	19		68
12	17					17												15			68
13						13				20		24	3				13	4			67
14									7										5		67
15	5					27							20		12				16		69
16	21		28							19	23	27				16		22	15		69
18			25			12				15	5		22					20	15		66
20																11					68
21	16		24			18			6	4								18			68
23			7							13								16			66
26	28		9			30				24			22			22	3	41			67
28	5		3			9			2								5	14			69
30	22					19			7	21	4	13		14				20	31		69
1923																					
Jan. 2	32					14							25	5		6	19	30			71
3				5		3															68
5						17								10			15		6		69
8												20		20			20				67
9						16												15			69
10						15				7		18	21				16	15			66
11	17	2	1			9								19				9	20		66
12												25				17	29	17			67
13										11									8		68
14						24							20								62
15	21					26						13		19			21		6		66
16	17									16		15			5		18	12			67
17	6										(b)			8	12				20		66
18	8									21			16	15				14			69
19	6					24			6	3							39		26		72
20						8												8			70
22	14						10		6			20	13				22				67
23	18					25				13		6		14							70
25		10				9				12		17		37		6	8	12			72
26						6				8		8	13						23		72
27										15											71
29										11							12	22	17		68
30	8											14						6	8		68
31	11					10				18					10			16			69
Feb. 1	14	5													4		7				70
2						7				39		19	14	5		11	24				69
3													12	9	4		14	21			71
5						6				19											66
6												10					18				69
7	7					6	4								3			11			71
8										8				9			4	15	8		70
9										8		21			10						70
10												17					2	19	5		67
12	17	42				3						45	22	12							66
13					5		39			15		14		10				5		11	70
14	7	5								17		48			23						68
15							40		5					8						11	69
16		20				5			5			33	16	6				7			65
17	23				7									5	20	9				8	69
18							19														64
19		29					7			12		67			12					3	68
20	14	5				5	4									8		5	8		70
21	18								4			25		9	16						67
22							28			23									27		66
23	20					6						12		20	29						67
24									31								(c)				64
25																39			44		68
26							15					(c)			5				16		70
27	9						27			3				15	17	6		5			70
28	6																		7		70

(a) Male escaped. (b) Female died. (c) Male died.

TABLE III.—*Oviposition of Necrobia rufipes*—Continued

Date of oviposition	Eggs laid by female of pair No.																			Daily mean temperature
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	°F.
1923																					
Mar. 1	6												6						13		68
2												5	6		26				8		68
3	7	13			9							13		6		26					73
4	14																				73
5					6					26			18	6							71
6		40													13				25		69
7		34			5		7		20				11		24						71
8							3		5					6	7				5		69
9	10						7		32			4							7	6	70
10										25											68
11	17																				62
12	6	32					17								16	21			14		71
13					19		19		23			6							31		74
14					7				23							14			9		71
15	12	50			4				14	20		13			18				12	7	69
16							12					21			8	2			4		68
17		39			3				21				13			5			11	6	68
18									20												61
19	7	47			14							45	19		36					28	66
20							5													6	68
21									17												70
22	2									8					44				22	18	73
23							19														75
24		67							38	34		16			7				43	28	74
26				(a)					8	15		4			7				(a)		66
27		50													19	20				17	68
28									6												71
29										19		6	18								69
30									24												69
31																				2	71
Apr. 1										3											56
2		6			16										42						71
3		42			1		8					8				8					72
4					15											9				12	74
5	9																				73
6	4														50						73
7		34					23		5			26								6	73
9									24												68
10		51			11				7	6		3	9		9	20				9	69
11	16								3												69
12									14	19			18							24	71
13									19												72
14	14								5	21			24			9					69
16					28		7		26	10										37	68
17																8					70
18	15								16			10			9						71
20			(b)		52					10			20								73
21	16				10				28				15							32	75
23		24	(a)							30			8		45						74
24					19			2													65
25					12		5	3							8	12					71
26							(a)	3					17							18	71
27												14									71
28		6					7						16		45	28		(b)		30	74
30		52							27	25						16				47	68
May 1	(a)				32										11					5	70
2	15				14				10											13	71
3		20			27															28	71
5					7			4		19					11	22					74
7						(b)		7		10		11			53					50	73
8					6											6					73
9					20				4	4						16					71
10										3										7	66
12						(a)			(a)				(a)							25	71
14															7	27					72
15																				15	74
16								5	7	9										8	78
17					9					15						34				24	76
18					12											5					73
19					24			14		12					4	22					76
20											(a)										76
21								34		8						19					76
22		(b)													25					23	75
23					11			12								10				20	71
24					13			22		20					26	35				11	72
25					20					12					12	19				5	73
26					17			12		9					7					6	74
28					6			22	13						26	40				36	80
29								11							11	7				7	82
31				(b)	12											12					75

(a) Male died. (b) Female died.

TABLE III.—*Oviposition of Necrobia rufipes*—Continued

Date of oviposition	Eggs laid by female of pair No.																				Daily mean temperature
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1923																					
June 1												(a)									77
2					11										27	42				18	84
4								17		11										15	85
6					20			7		23				(a)	11	22					85
7					43			7		5					(b)	24					84
8					4					11						23				27	81
9					22			24							43	31					75
11					30			26							22	48				29	76
12								16													72
13																24					71
14					11										10	9					72
15					40			11								57				51	78
16					12			10							32	12					79
18					18			24							33	64				34	83
19					10										10	6					84
20					17			22	(a)	(a)						44					87
21					23			15								24					89
23					27			11								51					85
24																				(a)	86
25					26			23								37					88
26					13			11								13					90
28					8			30								33					79
29					13																74
July 2					14		(a)	(b)								28					73
3					40																75
4					(b)			10													78
5																35					81
6								17													82
8					20			6													79
10					12											16					79
11					12			15								45					81
12					16																80
13								17								44					79
16					42			19								43	(a)				82
18					11			20								11					79
20					13			20					(a)			26					81
21					4											8					85
24					10			27								45					73
25																8					76
26								19								16					76
27					22			4								29					77
28																23					80
30								48								18					80
Aug. 1								30													69
2								17								20					72
3					15			27								16					80
4					11			27								38					81
6					26			108								53					84
7								31								13					85
8								37								41					86
9								30								20					78
11								43								32					78
13					5			40								33					79
14								12								14					76
15								8													80
16					5			24								16					77
17					9			28								18					72
18								15													71
20					25			38								19					78
21					7											5					78
22								29								4					75
23					11			25													69
25								19													73
27					6			28													75
30					5			17													75
Sept. 1					22																76
4								42													80
6					8			20													72
10																(b)					69
18								43													67
24					(a)											(a)					78
26								51													76
28								12													75
Oct. 30								(a)													
Nov. 13																			(a)		
20										(b)											
Total	846	1316	1087	301	1197	1088	481	1497	583	1029	576	854	744	598	1042	2131	351	876	706	823	

Average eggs per female 906. Date of death of female No. 15 not recorded.

(a) Female died. (b) Male died.

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In Table III the conspicuous features are the large numbers of eggs laid by several of the females, the length of the records, the marked differences in fecundity of the females, and the long rest periods during which no eggs were laid. As the present report appears to be the first detailed account of the biology of a representative of the large family Cleridae, there is no opportunity for comparison of the life history of *Necrobia rufipes* with an allied form.

As shown by Table III, mated females continued to lay eggs for several months after having been deprived of the male through its death or escape. The male of pair No. 14 escaped December 5; eggs laid by the female January 18 and March 5 produced larvae. As previously noted, eggs laid by old females not infrequently collapse and fail to hatch. This occurred, for example, with the eggs of pair No. 2, which were laid April 23 and May 3, and the eggs of pair No. 9, laid on May 2. The records detailed in Table III are summarized in Table IV.

TABLE IV.—*Oviposition and longevity of Necrobia rufipes, 1922 and 1923* [a]

[Food: Larvae of *Piophila casei*]

Pair No.	Male and female emerged and mated	Longevity of male	Longevity of female	Duration of period, mating to oviposition	Duration of period of oviposition	Duration of period, end of oviposition to death of female	Total number of eggs laid
	1922	*Days*	*Days*	*Days*	*Days*	*Days*	
1	Sept. 1	242	[b]	7	236	[b]	846
2	Sept. 6	375	258	2	237	19	1,316
3	...do....	229	226	3	124	99	1,087
4	...do....	201	267	2	119	146	301
5	Sept. 11	296	378	155	205	18	1,197
6	Sept. 12	242	237	5	159	73	1,088
7	Sept. 16	222	289	4	221	64	481
8	...do....	289	409	8	369	32	1,497
9	...do....	238	277	58	196	23	583
10	...do....	430	277	28	237	12	1,029
11	Sept 18	234	121	4	99	18	576
12	...do....	161	256	20	211	25	854
13	Sept. 19	235	304	6	215	83	744
14	...do....	[b]	260	17	153	90	598
15	Sept. 20	229	------	30	------	------	1,042
16	Sept. 21	354	368	12	323	33	2,131
17	Oct. 6	131	283	22	43	218	351
18	Oct. 16	325	194	2	132	60	876
19	Oct. 20	157	389	8	147	234	706
20	Oct. 21	248	246	113	127	6	823

[a] This table is a summary of the records detailed in Table III. Compare with Table II.
[b] Escaped.

DEVELOPMENTAL PERIOD

In the first rearing trials larvae were provided with what appeared to be the preferred food—stale bacon. This was heated to about 120° F. for a day or two in order to extract some of the low-melting-point fat, which was so abundant in unheated pieces of bacon that the larvae were often smothered in it. Although larvae usually become large and vigorous when they develop in whole hams, shoulders, or sides of bacon, the mortality in vials containing small pieces of the same meat was high and development slow, frequently resulting in dwarfed individuals. The results of the rearing experiments in which stale fat bacon was used are assembled in Table V.

Trials shown in Table V were preliminary, but the results, compared with those given in Tables VI and VII, show that an exclusive diet of smoked pork is not the most favorable food for larvae reared in close confinement.

In some of the rearing work done subsequently to that shown in Table V the ham-beetle larvae were fed only skipper maggots. Newly hatched larvae were transferred to Petri dishes, 10 or 20 to a dish, where they were given crushed skippers until nearly full grown, when live skippers were provided. The mortality in these dishes was nearly always high. There was probably cannibalism, and the soft posterior proleg of the very young larvae often stuck to the smooth glass, resulting in their death by starvation.

Records of the development of larvae fed with skippers are given in Table VI. A comparison of this table with Table V shows the advantage to the insects, when closely confined as larvae, of a diet of maggots in the place of stale bacon.

In Table VI the shortest developmental period is shown to have been 30 days, including 17 days as a growing larva and 13 days within the cocoon as larva, prepupa, pupa, and adult. According to the results included in Table I the incubation period may be as short as 4 days; the minimum preoviposition period recorded (Table IV) is 2 days. The life cycle, therefore, is possibly as short as 36 days.

The details of the larval life are given in Table VII. In this set of experiments individual larvae were reared in large veterinary capsules, as explained previously under "Larval behavior." The larval stage is divided into three, sometimes four, instars.

PUPAL PERIOD

Details as to the duration of the pupal period and the prepupal period within the cocoon were secured both by opening cocoons and by examination of the insects in cells that had been built against the glass of the vials. In general, the prepupal stages (resting larva and prepupa) occupied about the same time as the pupal stage. Table VIII gives the results of these examinations.

TABLE V.—*Development of Necrobia rufipes, 1922*

[Larval food: Stale fat bacon]

Date eggs were laid	Number of eggs in vial	First cocoon formed	Emergence began	Minimum period, egg deposition to cocoon formation	Minimum cocoon period [a]	Minimum period, egg deposition to adult emergence	Average daily mean temperature during egg to adult period
				Days	*Days*	*Days*	° F.
Mar. 22	28	.....	Aug. 17	.....	.....	148	75
24	5	.....	July 14	.....	.....	112	74
24	25	.....	Sept. 3	.....	.....	163	75
24	21	.....	July 31	.....	.....	129	75
26	33	.....	July 11	.....	.....	107	74
30	33	.....	July 9	.....	.....	101	74
30	11	.....	Aug. 31	.....	.....	154	75
30	3	July 20	(b)	112	.....	(b)	.....
Apr. 1	9	.....	July 9	.....	.....	99	74
1	8	.....	...do.....	.....	.....	99	74
9	26	July 3	July 18	85	15	100	75
9	3	July 15	July 29	97	14	111	76
11	4	July 28	Aug[2] 9	108	12	120	76
15	11	Aug. 15	Sept. 7	122	23	145	76
19	7	July 15	Aug. 4	87	20	107	76
19	11	July 24	Aug. 17	96	24	120	76
28	8	July 12	July 27	75	15	90	77
May 5	24	Aug. 5	Aug. 31	92	26	118	77
13	13	Aug. 3	Aug. 26	82	23	105	77
13	13	...do.....	(b)	82	.....	(b)	.....
16	10	July 20	Aug. 26	65	37	102	77
June 21	11	Aug. 3	...do.....	43	23	66	78
Total	.....	.....	.....	1,146	232	2,296	.....
Average	.....	.....	.....	88	21	115	.....

[a] The figures in this column represent probable but not actually known minimum cocoon periods. In each vial several cocoons were formed in the cotton plugs, and the first adult to emerge did not necessarily come from the cocoon which was formed first.

[b] No emergence.

OVERWINTERING

In an experiment made by the writers to determine the ability of full-grown larvae to survive 48° to 50° F. in a refrigerator, five out of seven lived about six months and one survived for seven months.

On December 21, 1922, an old shoulder infested with adults and various sizes of larvae was exposed to outdoor temperatures. The meat was kept in a fumigating box, protected from rain and snow, until March 24, 1923. After three months of winter temperatures, all adults and small and half-grown larvae were found to be dead, and many full-grown larvae were dead. Seven full-grown larvae survived, however, and eventually transformed into adults.

From these records, full-grown larvae appear best able to withstand such winter temperatures as obtain at Washington.

CONTROL SUGGESTIONS

Riley (*24*) recounted the control measures used by a concern in St. Louis. This firm dipped wrapped hams in a mixture of flour, water, a little glue, and chrome yellow, sometimes with "heavy spar" (barium sulphate) added. He suggested that a heavier canvas be used and applied before the first of May. Following the adoption of Riley's suggestions, losses were practically eliminated.

It should be observed that these suggestions were made in the days before artificial refrigeration was widespread, when hogs were necessarily slaughtered in the cool months. Practically all pork for use in summer had to be cured or smoked, or both, during

TABLE VI.—*Development of Necrobia rufipes, 1922* [a]

[Food: Mature larvae of *Piophila casei* L.]

Date egg hatched	Larval period	Cocoon period	Hatching to emergence	Date egg hatched	Larval period	Cocoon period	Hatching to emergence
	Days	*Days*	*Days*		*Days*	*Days*	*Days*
Sept. 5	28	13	41	Sept. 23	25	16	41
5	34	11	45	23	28	15	43
5	35	10	45	23	28	15	43
5	34	14	48	23	28	17	45
5	34	15	49	23	37	15	52
5	35	14	49	23	42	16	58
5	35	14	49	29	24	15	39
5	35	15	50	29	26	14	40
5	41	12	53	29	42	15	57
5	56	16	72	Oct. 2	28	14	42
5	80	19	99	2	28	15	43
12	30	14	44	2	28	17	45
12	30	14	44	3	23	18	41
12	30	15	45	3	41	26	67
12	30	16	46	3	41	29	70
12	36	13	49	3	55	18	73
12	36	13	49	4	26	15	41
12	36	14	50	4	30	17	47
12	36	15	51	4	30	18	48
12	37	14	51	4	48	16	64
12	36	15	51	4	35	52	87
12	36	15	51	6	38	16	54
12	36	15	51	6	41	18	59
12	36	16	52	6	46	16	62
12	36	16	52	6	41	21	62
12	36	16	52	6	46	16	62
12	37	15	52	6	46	17	63
12	36	16	52	6	48	16	64
12	39	13	52	6	46	18	64
12	36	17	53	6	48	18	66
12	39	15	54	6	46	20	66
12	41	15	56	6	48	19	67
12	43	14	57	6	48	20	68
12	44	14	58	6	48	20	68
12	45	17	62	6	48	21	69
12	48	16	64	6	52	19	71
12	48	16	64	6	52	22	74
12	59	16	75	6	52	29	81
12	59	18	77	7	45	16	61
12	65	16	81	7	45	16	61
20	28	13	41	7	45	16	61
20	28	13	41	7	45	17	62
20	28	17	45	7	45	18	63
20	35	19	54	7	47	16	63
20	40	14	54	7	47	18	65
20	54	16	70	7	47	18	65
21	25	11	36	7	45	24	69
21	25	12	37	8	19	17	36
21	25	13	38	11	19	15	34
21	25	14	39	11	38	7	45
21	32	17	49	11	28	24	52
21	32	18	50	11	49	20	69
23	17	13	30	11	49	22	71
23	17	13	30	12	26	15	41
23	23	8	31	15	22	14	36
23	19	13	32	15	39	15	54
23	23	12	35	15	37	17	54
23	23	12	35	15	39	19	58
23	19	16	35	15	47	21	68
23	23	13	36	15	43	29	72
23	23	15	38	15	54	19	73
23	23	15	38	17	35	17	52
23	23	15	38	18	40	22	62
23	25	14	39	20	47	22	69
23	23	16	39	26	36	27	63
23	28	12	40	26	43	20	63

[a] Larvae reared in Petri dishes. Sept. 5 to 26, exposed to laboratory temperatures, average daily mean 76° F.; Sept. 26 to Nov. 3, in incubator at 80° to 85° F; Nov. 3 to Dec. 8, temperatures not recorded but were usually between 70° and 80° F. All cocoons incubated at 80° to 85° F.

the season of low temperatures and stored for future consumption.

According to Perkins (*23, p. 126*) the larvae do not seem to be able to eat their way through any covering, and thoroughly wrapped hams are not therefore exposed to injury.

In the Philippines this species is controlled in copra by fumigation under a tarpaulin with carbon disulphide (*10*).

Herrick (*14, p. 280*) stated that the injured parts of infested meats can often be cut away.

Ashbrook, Anthony, and Lund (*3, p. 25–26*) have given an important control measure, which consists of removing the original string from smoked meats before wrapping them and tying a new string tightly around the outside of the package. This is necessary because it is impossible to make an insect-tight package if a string passes from the meat through the wrappings.

Ordinarily there appears to be slight danger of infestation of newly smoked pork products by ham beetles. If meats are wrapped promptly and tightly, there should be no trouble, but long-stored hams and sides of bacon that are hung up unwrapped or packed in crates accessible to the beetles are likely to become infested during warm weather.

The ham beetle is able to find an entrance to meat which is apparently tightly covered, and in view of the insect's ability to reach smoked meats protected by several overlapping layers of paper it is hardly to be wondered at that the theory of abiogenesis has been used to explain the presence of purple worms in these products.

Careful screening with fine wire cloth (30 meshes per inch), as recommended to exclude the cheese skipper, will also be effective against ham beetles.

Reconditioning by trimming off infested parts with a knife is sometimes resorted to, especially with bacon. The eradication of an infestation will be hastened if the meats and rooms in which they have been stored are fumigated with hydrocyanic-acid gas. The use of this gas for the fumigation of meats has been approved by the Bureau of Animal Industry, United States Department of Agriculture (*33*).

TABLE VII.—*Development of individual larvae of Necrobia rufipes*

[Food: Eggs of *N. rufipes* followed by larvae of *Piophila casei*]

Date egg hatched	Duration					Date egg hatched	Duration				
	First instar	Second instar	Third instar	Fourth instar	Larval stage		First instar	Second instar	Third instar	Fourth instar	Larval stage
1923	*Days*	*Days*	*Days*	*Days*	*Days*	1923	*Days*	*Days*	*Days*	*Days*	*Days*
May 13	8	6	10	13	37	May 15	9	6	10	.....	25
13	10	6	13	.....	29	15	7	8	16	.....	31
14	7	8	15	.....	30	16	6	8	19	.....	33
14	11	4	10	11	36	16	6	8	8	.....	22
14	7	8	8	10	33	16	6	8	8	11	33
14	7	8	8	16	39	16	6	8	19	.....	33
14	9	6	10	8	33	16	6	6	10	.....	22
14	9	6	21	.....	36	16	6	8	19	.....	33
14	9	6	21	.....	36	16	6	8	8	.....	22
14	9	8	19	.....	36	16	8	6	22	.....	36
15	7	8	8	13	36	16	8	6	16	.....	30
15	7	6	24	.....	37	16	6	8	10	.....	24
15	7	8	10	.....	25	16	8	8	14	.....	30
15	7	8	10	.....	25	16	6	8	10	12	36
15	9	6	22	.....	37						

NOTE.—Mean temperatures (°F.): May 13, 73°; May 14, 72°; May 15, 74°; May 16, 78°; May 17, 76°; May 18, 73°; May 19, 76°; May 20, 76°; May 21, 76°; May 22, 75°; May 23, 71°; May 24, 72°; May 25, 73°; May 26, 74°; May 27, 78°; May 28, 80°; May 29, 82°; May 30, 77°; May 31, 75°; June 1, 77°; June 2, 84°; June 3, 86°; June 4, 85°; June 5, 87°; June 6, 85°; June 7, 84°; June 8, 81°; June 9, 75°; June 10, 76°; June 11, 75°; June 12, 72°; June 13, 71°; June 14, 72°; June 15, 78°; June 16, 79°; June 17, 80°; June 18, 83°; June 19, 84°; June 20, 87°; June 21, 89°; June 22, 89°.

SUMMARY

The ham beetle (*Necrobia rufipes* De G.) is widely distributed over the warmer parts of the world, frequently being found as an inhabitant of stores of bones and other inedible animal products. Among the islands of the Pacific it is a pest of copra. In parts of the United States sporadic injury, occasionally of considerable extent, is done to long-stored hams, shoulders, and bacon.

The adult is a shiny, green beetle 3.5 to 7 mm. long; the larva is purplish and about 10 mm. in length when full grown. Both imago and larva are

active and predatory; their habit of infesting smoked pork has probably been acquired rather recently. The larvae are responsible for most of the damage to infested meats. They bore holes in the meat, preferably burrowing into the fat parts. The adults feed chiefly on the surface. Pupation occurs within a white cocoon constructed with drops of froth emitted from the mouth of the larva.

The adult may live for more than 14 months, the female depositing as many as 2,100 eggs during that time. In warm weather the incubation period is four days or more. The preoviposition period is as brief as two days. The period from hatching of the egg to adult emergence may be as short as 30 days, including 17 days as growing larva and 13 days within the cocoon.

An important consideration in the prevention of injury by the ham beetle is the careful wrapping of meats. Probably the most effective method for the eradication of an infestation is a thorough fumigation with hydrocyanic acid gas.

TABLE VIII.—*Cocoon period of Necrobia rufipes, 1922*

Cocoon formed	Prepupal period in cocoon [a]	Pupal period	Cocoon period	Average temperature during cocoon period	Cocoon formed	Prepupal period in cocoon [a]	Pupal period	Cocoon period	Average temperature during cocoon period
	Days	*Days*	*Days*	°*F.*		*Days*	*Days*	*Days*	°*F.*
Jan. 17	----------	----------	42	67	Aug. 21	17	4	21	76
17	----------	----------	46	67	21	10	12	22	76
17	----------	----------	55	67	21	9	14	23	76
18	----------	----------	51	67	21	15	8	23	76
19	----------	----------	45	67	21	8	18	26	76
20	----------	----------	47	67	21	5	22	27	76
20	----------	----------	55	67	21	9	34	43	73
21	----------	----------	41	67	21	26	54	80	71
21	----------	----------	47	67	22	10	10	20	76
22	----------	----------	40	67	22	17	31	48	73
22	----------	----------	52	67	23	16	4	20	77
24	----------	----------	36	67	23	16	6	22	76
24	----------	----------	42	67	23	14	10	24	76
24	----------	----------	44	67	23	14	10	24	76
24	----------	----------	45	67	23	14	12	26	76
24	----------	----------	46	67	23	16	12	28	75
24	----------	----------	49	67	24	13	5	18	76
26	----------	----------	46	67	24	15	8	23	76
27	----------	----------	41	67	24	14	9	23	76
28	----------	----------	41	67	24	13	12	25	76
28	----------	----------	58	67	24	13	13	26	76
Feb. 4	----------	----------	46	66	24	15	15	30	75
Mar. 25	----------	----------	32	69	24	19	23	42	73
25	14	21	35	69	24	17	27	44	73
26	12	21	33	69	25	14	8	22	76
26	12	28	40	69	25	15	7	22	76
27	12	21	33	69	25	13	9	22	76
28	12	24	36	69	26	13	8	21	76
31	10	21	31	69	26	12	15	27	75
Apr. 1	10	22	32	69	26	15	13	28	75
3	9	24	33	70	28	9	10	19	76
9	9	21	30	70	28	9	13	22	76
Aug. 14	7	4	11	77	28	13	11	24	75
15	4	9	13	77	28	12	30	42	73
15	9	12	21	76	29	8	10	18	77
15	10	11	21	76	Sept. 1	10	8	18	76
15	14	8	22	76	2	6	8	14	77
16	6	11	17	76	2	8	22	30	73
16	9	12	21	76	2	9	23	32	73
16	13	9	22	76	5	6	9	15	75
16	12	11	23	77	5	6	21	27	72
17	11	5	16	76	5	6	21	27	72
17	11	9	20	76	6	14	13	27	72
17	11	9	20	76	14	22	12	34	70
19	8	11	19	76	14	17	19	36	70
19	8	14	22	76	16	15	15	30	70
19	17	7	24	76	16	14	18	32	70
19	19	9	28	76	16	21	11	32	70
19	17	11	28	76	16	16	19	35	69
21	15	5	20	76	16	32	22	54	68
21	10	11	21	76					

[a] The prepupal period (period from formation of cocoon to appearance of pupa) and pupal period occurring in cocoons formed Aug. 14, et. seq., were determined by opening the cocoons. Prior to Aug. 14 the same notations were made by observations through the glass of vials containing cocoons built against the glass.

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STUDIES ON CONFORMATION IN RELATION TO MILK PRODUCING CAPACITY IN CATTLE[1]

IV. THE SIZE OF THE COW IN RELATION TO THE SIZE OF HER MILK PRODUCTION[2]

By JOHN W. GOWEN

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Woll (*9*, *10*)[4], Peters (*8*), Grady (*4*), Nevens (*7*) reanalyzing Woll's data, and the work of several European workers have shown that as the weight of the cow increases, her yield of milk and milk solids increases. This fact holds for dairy cattle in the pure breeds and also in the dairy grades. The work of these writers further shows that the economy of production within any one breed is greater for the large than for the small cow, because of the fact that the small cow needs proportionately more feed to maintain her body weight. This difference in the requirements of the larger and the smaller cows has been interpreted as due to the difference in the surface areas of their bodies. Thus, while the surface area of the larger cows is greater than that of the smaller cows, proportionally to their weight the surface area of the bigger animals is much less. As heat radiates from the body surface, the food units necessary for the larger cows would be proportionally less per given unit of body area. As the cow's capacity to handle quantities of food increases with her size, the amount of food left for milk production after maintaining the body weight is proportionally greater for the large than for the small cow.

Further research may show these assumptions to be not entirely correct. Harris and Benedict (*5*) have shown that the surface-area formula for basal metabolism in men and women is not so accurate as a height, weight, and age formula. There may well be other factors of deeper physiological significance which really govern the basal metabolism of the animal, factors which in our crudeness we are interpreting as causative when they are only correlated with the basic factor. Be that as the future may show, it is of interest to extend the work of these investigators to other measures of size in cattle.

The Holstein-Friesian Cattle Club (*6*) in the early history of their Advanced Registry took a rather extensive series of measurements on their cattle. There are 385 of these records, the cows ranging in age from 1 year and 6 months to 10 years and 6 months. All records for measurement were taken within the year in which the 7-day lactation record was made. These records had measurements on height at shoulders, height at hips, body length, rump length, body width, thurl width, and body girth. The weight, either actual or estimated, was given in 339 of them. All records contained the 7-day milk yields and butter-fat percentages. The age also was recorded when the test was made. The method followed in making the measurements herein recorded conform to the following rules:

> The animal must be brought to stand on a level place and in a natural position, the feet squarely under the body, the head at a medium height, and the neck straight. The two items of height are taken perpendicularly from the ground to the top of the animal, the one immediately over the knee and center of the shoulder, and the other over the hook bone to center of the back; the length of body is taken from the extreme front of the shoulder point to the extreme rear and highest point of the rump, diagonally in a straight line; the length of the rump, from the extreme front side of the hook bone to the extreme of the rump as described above; the width of the hips, from the outside of one hook bone to the outside of the other in a straight line; the width of the thurl from the outside of one thurl bone to the outside of the other, also in a straight line; the girth by a tape closely fitting the smallest circumference of the chest; in the latter measurement the tape must be drawn so closely that a slight movement of it will move the skin of the animal. If the head is lowered from the natural position, this measure-

[1] Received for publication July 14, 1924; issued June, 1925.

[2] This paper is one of a series of investigations in animal husbandry, the continued prosecution of which has been made possible by a grant to the author from the Rockefeller Institute for Medical Research.

The data for this paper are taken from the Holstein-Friesian Advance Registry records. They include records for the 7-day milk yield, age of the cow at time of test, height at shoulders and hips, body and rump length, body and thurl width, girth, and weight. All measurements are taken according to the rules prescribed by the association and by men designated as inspectors.

[3] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 168.

[4] Reference is made by number (italic) to "Literature cited," p. 869.

ment will invariably be erroneous. And if the neck is turned to the right or left, the measurement of length of body will also be erroneous. Then follows the ascertaining of the weight, which must invariably be obtained by reliable scales; the date of service is then ascertained from the owner and the condition described. The date of the examination should also be recorded in some convenient place in the memorandum book.

The necessary analyses of these data show the averages and coefficients of variation seen in Table I.

The averages for age, butterfat percentage, and milk yield show that this group of cows is younger, has a higher butter-fat test, and is less productive of milk than are the cows of the whole Advanced Registry.

The series of means of the different parts of the body gives an idea of what might be called the "average" Holstein-Friesian cow. This average cow may or may not be a desirable specimen. It would be interesting to see a reconstruction based on these measurements. It might be found that the average is disproportionate for some of the parts, as was the case for the typical American soldier.

The weights of the cows are either estimated or actual. There are 161 records of actual weight and 178 of estimated weight. The actual weights have a mean of 1,078, a standard deviation of 162, and a coefficient of variation of 15.0. The distributions are consequently closely similar to each other.

The measurements of this average cow are, on the whole, quite a little less than those of the present Holstein-Friesian ideal. Thus in shoulder height the ideal is nearly 2 inches taller, in body length nearly 10 inches longer, in rump length 2½ inches longer, in body girth about 15 inches greater, in hip width 2 inches greater, in thurl width 2½ inches larger, and in weight about 200 pounds greater. Only in hip height do the average and ideal correspond.

The variation of the different parts of the cow is of considerable significance. The characters used in scorecard judging are essentially divisible into two classes, physiological and morphological. The writer has studied the records of over 1,600 score cards taken by more than 200 judges on Jersey Registry of Merit cattle (*1*, *2*). The judges who made these scores have undoubtedly taken cognizance of the increases in size of the parts scored as age advances, and compensated for such increase in recording their judgment on the cows. The amount of variation would consequently be expected to be less than it would have been had age been left out of consideration. The data on the Holstein-Friesian cattle are actual measurements. In general, the comparison of these actual measurements with the scores shows that the scores of the judges on conformation are actually more variable than are the parts of the cows from which their scores are taken. In other words, the judgment by eye is, on the whole, less reliable than estimates based on the yardstick.

The comparable results found on other forms in conjunction with those found for these Holstein-Friesian cattle are worthy of record. The weight of these Holstein-Friesian cows has practically the same variation as the weight of men or of the domestic fowl. Shoulder or hip height in cattle is slightly less variable than length of the forearm or femur in man. Rump length, body girth, and body length are slightly more variable than stature in man.

Table II reveals several facts of no little importance in the selection of dairy cattle. Every one of the variables has a rather large positive relation to the quantity of milk secreted by the mammary gland. As shown elsewhere

TABLE I.—*Means, standard deviations, and coefficients of variation for the physical measurements of Holstein-Friesian cows*

Characters	Mean	Standard deviation	Coefficient of variation
Age	3.90±0.08	2.22±0.05	56.9±1.8
Milk yield	338 ±2	72.6 ±1.8	21.5± .6
Shoulder height	52.8 ± .1	1.94± .05	3.7± .1
Hip height	53.8 ± .1	1.91± .05	3.6± .1
Body length	62.0 ± .1	3.73± .09	6.0± .2
Rump length	20.4 ± .1	1.44± .04	7.0± .2
Body width	21.3 ± .1	1.72± .04	8.0± .2
Thurl width	19.0 ± .1	2.63± .06	13.8± .3
Body girth	73.2 ± .2	4.59± .11	6.4± .2
Weight	1088 ±6	164.0 ±4.2	15.1± .4

(*3*) a fairly large element in this correlation is the fact that the age of the cow materially influences her milk production and at the same time and in nearly like measure causes a growth in the size of the body parts. On the other hand, if one has little or no knowledge of the age of the cow and selects from a group the largest, for example, in body length or girth, one is quite sure of choosing the cow which is producing the most milk.

These facts are brought out graphically in Figure 1, which shows the average milk yield for the different classes of cows as indicated by body measurements. The size of the cows is judged by the size of the eight different measurements on the cow's body shown in Table II. The heavy lines of the figure represent the majority of the data. All the body measurements appear to be related to milk yield in a linear manner. The relation between milk yield and age appears to be linear up to about five years, when the average milk yield remains constant for the other ages. Two peculiar results are noticeable.

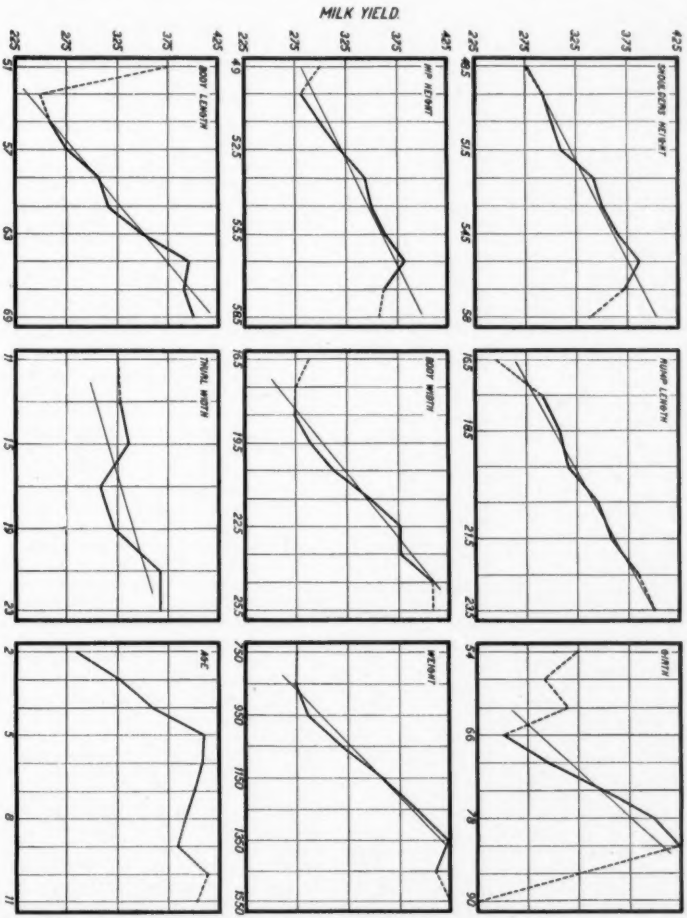

FIG. 1.—Relation of body measurements and age to the milk yield of the cow. The measurements are in inches, the weights in pounds, and the ages in years. Those points of doubtful significance, depending on less than 10 individuals, are shown in dotted lines.

TABLE II.—*Correlation of age and body measurements with the milk yield of the cow*

Characters	Correlation coefficient
Age	0.592±0.022
Shoulder height	.380± .029
Hip height	.344± .030
Body length	.590± .022
Rump length	.391± .029
Body width	.520± .025
Thurl width	.214± .033
Body girth	.476± .027
Weight	.652±[a].021

[a] The correlation for the 161 actual weights for milk yield with body weight is 0.623.

The graph showing the relation of milk yield and body length has as its first observation a cow of short body length but of relatively high milk yield. As this average is based on only one individual it can scarcely be significant except in showing the amount of variation which can occasionally be found when considering individual cases. The graph for girth shows similar irregularity. The drop from the high point of milk yield with a girth of 82 inches to the lowest point in milk yield with a girth of 90 inches is based on one individual. It seems highly probable that there was an error in this measurement. However, as this is not known, the data are used as given. The irregularity for the first part of this curve likewise is due to very limited data.

The prediction equations derived from the correlation coefficients and the means and standard deviations of the data are shown by the straight lines. It is evident that these straight lines follow the major portion of the data very well for all cases except those for girth and age. The equation for girth is, as noted, very materially influenced by the irregularities of the first three observations and also the last. If these observations should be discarded it would be found that the resulting equation fitted the data for 66 to 82 inches of girth almost exactly. In the case of age it has not seemed desirable to calculate the linear line, as it is well known that this line does not fit the relation between age and milk yield. It has therefore been omitted from the original chart. It might be said, however, that in attempting to obtain the relation between the body measurements for a constant age this linear line might be used, as it would correct for most of the variability caused by age. This method of correction is given elsewhere.

Judging from these graphs it seems entirely reasonable to use straight lines to represent the increase of milk yield for the increase in body size. The linear equations for these data as noted above have therefore been calculated. These equations are given as follows:

(1) 7-day milk yield=14.2 shoulder height−412.9
(2) 7-day milk yield=13.1 hip height−366.5.
(3) 7-day milk yield=11.5 body length−374.1.
(4) 7-day milk yield=19.7 rump length−63.5.
(5) 7-day milk yield=21.9 body width−128.3.
(6) 7-day milk yield=225.8+5.9 thurl width.
(7) 7-day milk yield=7.4 girth−201.5.
(8) 7-day milk yield=23.8+0.289 weight.

These equations are used for the straight lines of Figure 1. We may note certain of the significant points connected with them. Thus for each inch increase in height at shoulders there is an increase in 7-day milk yield of more than 14 pounds. For each inch of increased height at hips there results an increase of milk yield of 13 pounds For an increase of body length of 1 inch there results an increase of milk yield of 11.5 pounds on the average. An increase of 1 inch of rump length represents an increase of milk yield of 19.7 pounds and an increase of body width at hips of 1 inch increases the average milk yield of the cow 21.9 pounds. An increase in width at the thurl represents a lower increase in milk yield, for 1 inch increase in thurl width is only equivalent to 5.9 pounds increase in milk yield. An increase of 1 inch in girth represents an increase of 7.4 pounds of milk on the average for such cows. An increase of 1 pound of weight represents an increase of milk yield of nearly 0.3 pound of milk.

These results can not, of course, be compared directly, as the range of variation for the various body measurements is markedly different. If what might be reasonably considered the end points of these different measurements as indicated by significant data are taken, it is found that the body length, body width, girth, and weight are the items which caused the most marked increases in the 7-day milk yields of the cows, as the measurements are varied from the lowest to the highest in the breed. Closely following these are rump length, height at shoulders and hips. The thurl width is the least important measurement.

It is of interest to compare the amount of variation in the milk yields controlled by these different body measurements. It will be remembered that the reduction in standard deviation due to the making constant of

any given variable is equal to the original standard deviation minus the original standard deviation times the square root of 1 minus r square ($SD—SD\sqrt{1—r^2}$). When the height of shoulders is made constant, the variability in milk yield of Holstein-Friesian cattle is reduced 5.4 pounds. When the height at hips is made constant, the variability is reduced 4.4 pounds. When the body length is made constant the variability is reduced 14 pounds. A constant rump length reduces the variation in milk yield 5.8 pounds. A constant body width reduces the variation in milk yield 10.6 pounds. A constant thurl width reduces the variation 1.7 pounds. By making the girth constant the reduction is 8.7 pounds in milk yield. A constant weight reduces the variation in milk yield 17.5 pounds.

From these data it is noted that body length, body width, girth, and weight are the most important items in relation to milk yield and its variation. Weight is the most important single element, but is closely followed by the body length and body width. Were just the significant measurements for girth to be taken it would be found that this measurement is nearly if not as important as that of body length. The figures given in the above paragraph may be inverted and the results expressed in this way. For cows of a constant weight the 7-day Advanced Registry records of Holstein-Friesian cattle would vary only to 77 per cent of the extent to which they now do. If the cows were of a constant body length the variation in milk yield would be only 80 per cent of that now found. In the same way we could convert the figures for the other measurements on the size of the cow.

SUMMARY

The results show that, for these cows at average age of 3.9 years and a milk yield of 338 pounds for the 7-day period, the average measurements are shoulder height, 52.8 inches; hip height, 53.8 inches; body length, 62.0 inches; rump length, 20.4 inches; body width, 21.3 inches; thurl width 19 inches; body girth, 73.2 inches; and weight, 1,088 pounds. The variations of these measurements are comparable to those taken on the body parts of other animals. The coefficients of variation range from 3.7 to 15.1. The most variable part is weight. The least variable is height at hips.

All of these body measurements are related to milk yield, so that an increase in any one of them results in an increase of 7-day milk yield. The relation of all measurements to milk yield is linear for these data. The most important element predicting milk yield is weight. Body length, body width, or girth, closely follow weight in the accuracy with which they predict milk yield.

Increases of one inch produce an average increase in milk as follows: Shoulder height, 14 pounds; hip height, 13 pounds; body length, 11.5 pounds; rump length, 19.7 pounds; body width, 21.9 pounds; thurl width, 5.9 pounds, girth, 7.4 pounds. An average of 100 pounds in weight results in an average increase of 29 pounds of milk.

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NECROSIS, HYPERPLASIA, AND ADHESIONS IN MOSAIC TOMATO FRUITS[1]

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INTRODUCTION

The extremely severe streak or winter blight type of mosaic occurred in a greenhouse crop of Bonny Best tomatoes at La Fayette, Ind., during November and December, 1923, and an opportunity was afforded to make a preliminary study of the structural abnormalities exhibited by many of the diseased fruits. The disease was characterized by the destructive necrotic streaking and spotting of leaves, stems, and fruits described in a previous account (*12*, *p. 8*).[3] Every one of the more than 800 plants in the house also evinced on the young leaflets the typical dark green puffy areas on a lighter background, a reliable diagnostic feature of the mosaic type of disease. The mosaic nature of the disease was further verified by successful inoculation of healthy tomato seedlings with the juice of a young fruit by J. B. Kendrick in another house and by numerous cultural tests which proved the internal fruit lesions to be free from bacteria.

Hundreds of fruits of all sizes and ages showing a variety of responses to the disease were available for study, and unstained free-hand sections of fresh material, mostly from very young green fruits, revealed most peculiar abnormalities in the tissues. The sections were mounted in water and preserved by adding lacto-phenol (equal parts of phenol, lactic acid, glycerin, and water). Material was also embedded in paraffin, sectioned on the microtome, and stained with gentian violet, safranin and licht green, Haidenhain's iron-alum haematoxylin, and Ziehl's carbol fuchsin.

NORMAL ANATOMY OF THE FRUIT

An acquaintance with certain phases of the normal anatomy and development of the fruit is a necessary preliminary to a consideration of the abnormal. The mature tomato with its several-celled ovary and fleshy walls and its bulky axile placentae bearing the seeds embedded in a pulpy matrix of thin-walled parenchymatous tissue is familiar to all. A very young ovary about 4 mm. in diameter as shown in Figure 1, A, differs mainly in that the ovules more or less completely fill the locular cavity and are not embedded in a cellular matrix. During the early stages of enlargement of the fruit the placental tissue grows out between the ovules (fig. 1, B) to form the gelatinous matrix of thin-walled parenchyma which separates and finally engulfs the ovules and completely fills the locular cavity (fig. 1, C and D) by the time the fruit is about 10 mm. in diameter.

The placental matrix touches the inner surface of the fruit wall or pericarp and the surfaces of the radial locular partitions, but remains free from these surfaces as well as from the epidermis of each ovule (fig. 1, B, C, and D). The young fruit enlarges very rapidly under greenhouse conditions and hence is composed of very active meristematic tissue. Figure 1 shows that growth must progress with extreme rapidity in the placental matrix in the short period during which the ovary enlarges to 1 cm. in diameter. Later the epidermal cells of the seed coat elongate enormously to form a palisade layer, which touches but remains free from the placental matrix.

GROSS ABNORMALITIES IN MOSAIC FRUITS

One of the most conspicuous symptoms on mosaic fruits is the eruption of brownish, translucent, rather flat-topped blisters of various shapes and sizes as illustrated in Plate 1, B, and in a previous paper (*12*, *p. 8*). These may later be bordered by a shallow peripheral fissure. Large lesions of this type may eventually contain numerous cuticular fissures and form

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[3] Reference is made by number (italic) to "Literature cited," p. 888.

roughened buckskin areas on the fruit as described and illustrated by McKay (*18, p. 181*). Mosaic fruits are also characterized by more deep-seated lesions, producing an irregular pattern of hard, sunken, brown or black pockmarks, as shown in Plate 2, E and F. Ripening fruits often show rather extensive sunken granular areas which fail to color properly and under which the seed pulp or placental matrix tends to remain greenish.

Ovaries and very young fruits may show brown epidermal or deep-seated

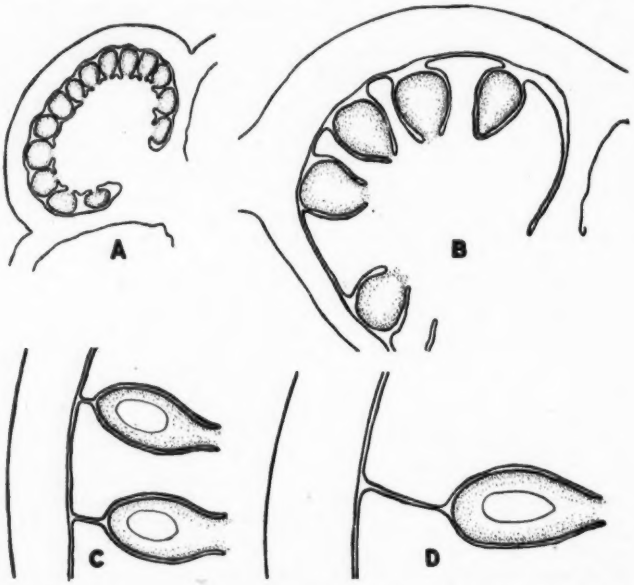


FIG. 1.—Origin and development of the placental matrix in which the seeds in a tomato fruit are embedded. Camera lucida diagrams drawn to the same scale (×15) to show how the placental tissue grows out between and around the ovules, finally engulfing them completely, during a very short period early in the growth of the fruit

A.—Locule of an ovary 3 mm. by 4 mm. in diameter showing ovules seated upon the surface of the axile placenta and completely filling the locular cavity

B.—Locule of a small fruit 5 mm. by 6 mm. in diameter showing how the placental tissue has grown out between the ovules into contact with the pericarp, thus surrounding and separating the ovules and filling the locular cavity as the latter enlarges. The ovules are receding from contact with the pericarp

C.—Portion of locule in fruit 7 mm. in diameter showing how the placental tissue has completely engulfed the seeds as the latter recede from actual contact with the pericarp. This placental tissue develops very rapidly and completely fills the enlarging locular cavity

D.—Portion of locule in fruit 10 mm. in diameter showing distance to which pericarp has grown from the seed, the intervening space being filled by a cellular matrix resulting from the outgrowth of the placenta. It is in this placental matrix that the abnormalities predominate in mosaic fruits. The growth of pericarp and seed may also be noted

EXPLANATORY LEGEND FOR PLATE 1

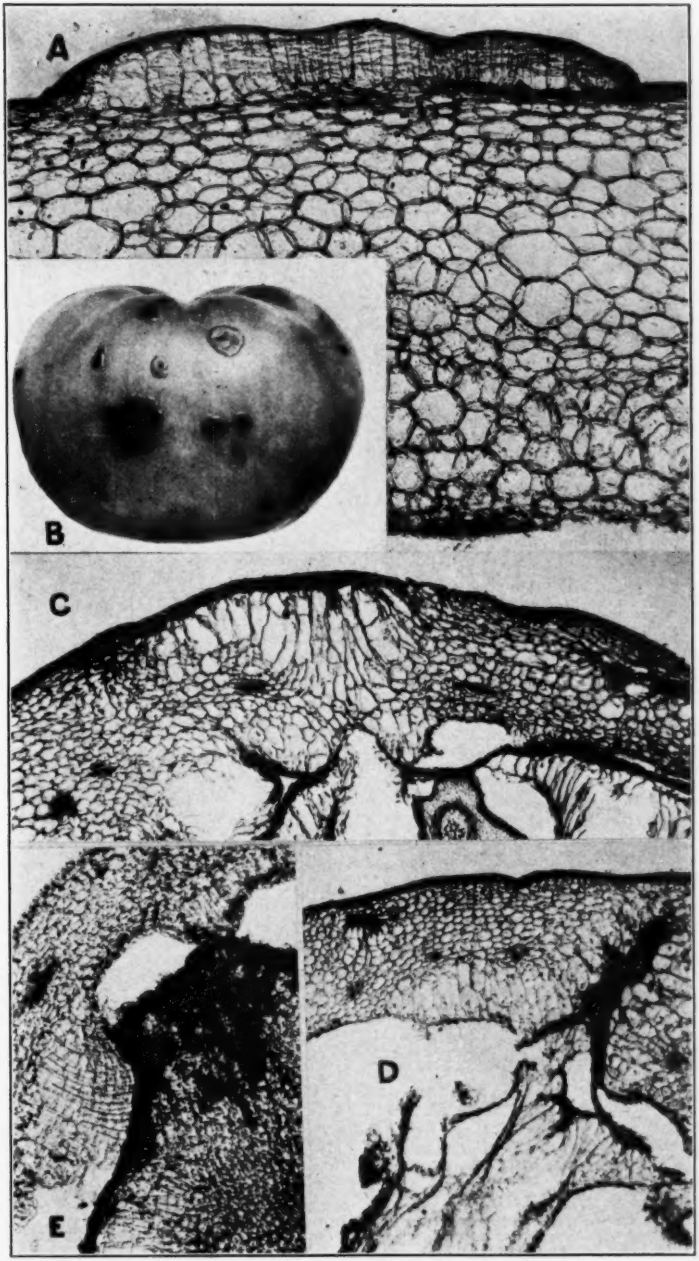
A.—Section of pericarp through a surface blister such as is illustrated in B, showing the hyperplastic character of the blister tissue. The subepidermal cells have elongated and divided, pushing up the epidermis. If this tissue collapses the blister sinks and the epidermis usually cracks where it is sharply curved upward at the edge of the blister, thus forming the marginal fissure noted about the older lesions. Photomicrograph × 33. Unstained

B.—Surface blisters on a mosaic tomato

C.—Section of a pericarp lesion resulting from necrosis and collapse of an area of subepidermal cells and hypertrophy of the cells immediately beneath the necrotic area. The necrotic surface of the underlying placental matrix, to which an ovule is adhering, is in turn adhering to the inner surface of the pericarp. Photomicrograph × 33. Stained with Haidenhain's iron-alum haematoxylin. Fruit 12 mm. in diameter

D.—Section of pericarp showing at the right a transverse necrotic plane accompanied by elongation of the near-by cells. At the left is a small necrotic spot in the pericarp. Photomicrograph × 33. Stained with carbol fuchsin. Fruit 12 mm. in diameter

E.—Intumescences from a radial locule partition extending into an abnormal cavity which has resulted from the collapse of the necrotic placental tissue. Photomicrograph × 46. Unstained. Fruit 7 mm. in diameter



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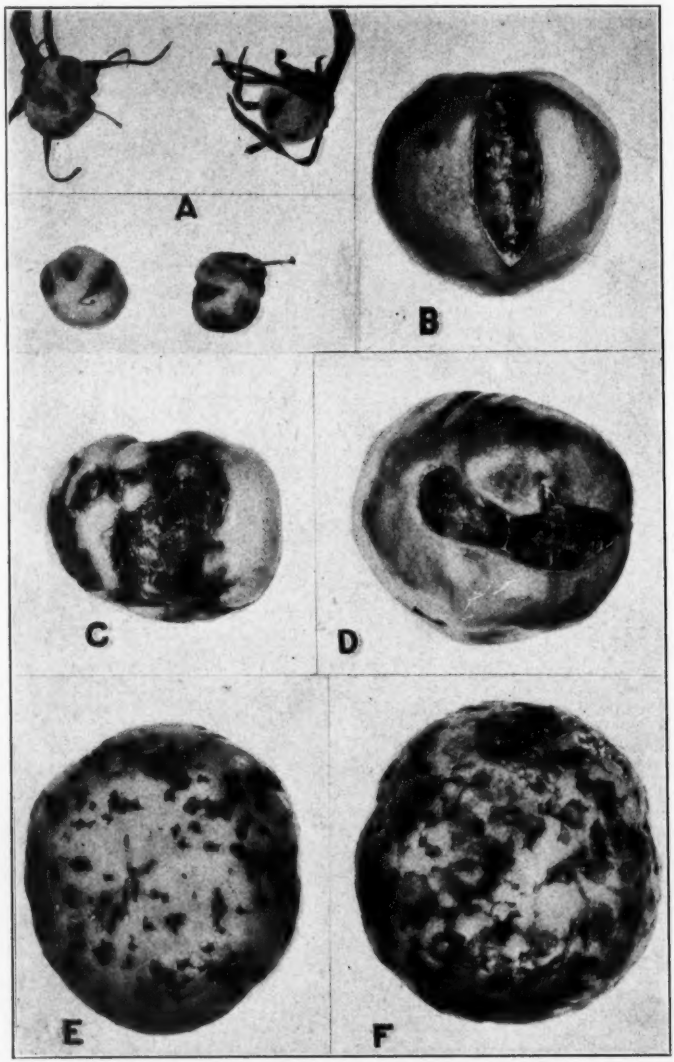


A.—Four very young tomato fruits showing necrotic lesions. Two show rupture of the pericarp and would probably develop into such fruits as those shown in Plate 3, C

B.—Longitudinal rupture of the pericarp, as a result of an earlier necrotic lesion, exposing a considerable area of the seed pulp or placental matrix

C.—Scabby lesions stunting growth and causing deformity and pericarp rupture in young fruit

D.—Transverse rupture of the pericarp as a result of an earlier necrotic lesion

E.—Scattered, slightly sunken surface lesions or pockmarks

F.—Coalescence of lesions producing a brownish pattern with a hard, sunken, granular surface. Such fruits fail to color properly

markings without the blister effect (pl. 2, A) and in some cases the entire surface of the fruit is involved, a condition associated with early abscission or invasion by rot-producing organisms. The discoloration may sometimes be internal and deep seated so as to show through the outer pericarp layers as an evident darkening of the underlying tissues (pl. 3, B).

The surface lesions may become hard, dark brown scabs which inhibit the symmetrical growth of the fruit and as a result produce marked malformation, as is shown in Plate 2, C. Often the brown necrotic lesions involve the pericarp so deeply that, after the affected tissues collapse, the weakened areas are ruptured by the growth pressure of the interior tissues. and large irregular openings are produced in the pericarp exposing the seeds and placental matrix to the drying effects of the air (pl. 2, B and D). In fruits thus affected when very young, as shown in Plate 2, A, mere shreds of the pericarp may remain as is shown in Plate 3, C, or deep cracks may result as shown in Plate 3, D.

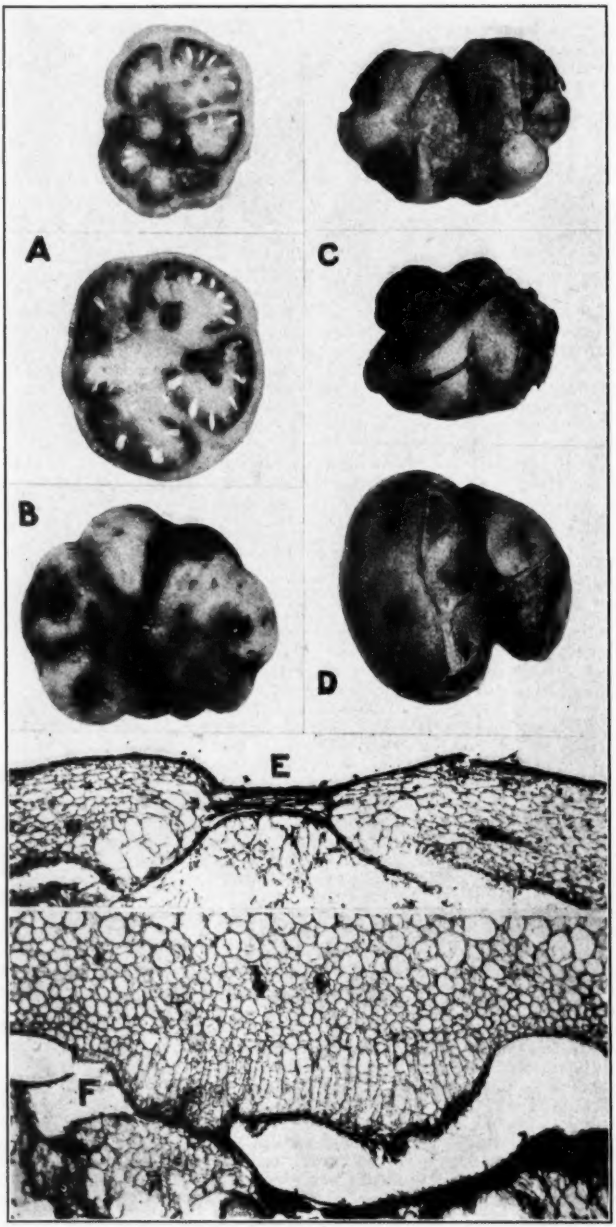
When the fruit is cut across it is found that brown necrotic regions usually occur throughout the interior tissues if there are any external lesions on the specimen (pl. 3, A) and in cases of extensive surface discoloration all or considerable portions of the interior may also be similarly discolored. Ordinarily, however, the internal necrosis occurs in the form of scattered strips, pockets, or thin layers, not continuous or connected with each other nor, as a rule, sufficiently extensive to completely inhibit the enlargement of the fruit, although its normal development may be seriously interfered with. The necrotic regions occur most abundantly in the placental matrix that fills the locules, and the most prevalent type consists of rather extensive necrotic planes parallel to and in rather close proximity to the locule walls. These necrotic regions are usually but not always surrounded or bounded by zones of rather firm, glassy or translucent tissue. In young ovaries many of the ovules are entirely necrotic and atrophied. This occurs usually in only part of the locules and often involves only part of the ovules in the locule. Many mature seeds show brown spots.

Examination of razor sections of the young fruits with a hand lens reveals very clearly the necrotic regions and the translucent zones associated with them and also shows rather extensive cavities associated with the necrosis of the distal portions of the placental matrix. These cavities may be entirely surrounded by a brown necrotic lining as is the small one in Plate 4, A, or may occur between the necrotic placental surface and the locule wall as in Plate 1, E. Furthermore many of the seeds may be displaced or abnormally located and oriented in the placenta, and the normal arrangement of the placental matrix as shown in Figure 1 is very generally interfered with to a greater or less extent. It is plainly evident also that necrosis often occurs within the seeds, that some ovules are affected more than others, and that various degrees of retardation in the development of the seeds and the placental matrix occur in the same fruit and even in the same locule. For example, certain locules in fruits 5 mm. and 7 mm. in diameter showed necrotic atrophy of the ovules and complete suppression of any development of the placental matrix beyond the stage shown in Figure 1, A.

NECROSIS, HYPERTROPHY, AND HYPERPLASIA

Microscopic examination of unstained sections shows that the surface blisters are produced by a compact muriform cushion of meristematic cells which have pushed up the epidermis (pl. 1, A). Epidermal pustules or intumescences of all sizes are found, the smaller ones being papillate rather than flat-topped. In the incipient stage of a blister, necrosis of the epidermal cells is visible and the hyperplasia is seen to originate in the first and second subepidermal cell layers (pl. 5, A). The translucent quality of the blisters is very evidently attributable to the lack of intercellular spaces in this hyperplastic tissue (pl. 5, B).

Zones of brown necrotic cells of varying shape, extent, and orientation are of common occurrence at varying depths in the pericarp, producing, as a result of collapse of the necrotic cells (pl. 6, B and C), dark sunken pockmarks on the exterior of the fruit. Such zones often involve the entire thickness of the pericarp and, as a result of early atrophy of the tissues (pl. 6, A) or collapse of the necrotic cells (pl. 3, E; pl. 6, C), produce weakened places in the pericarp which are easily ruptured by growth pressure. This results in such fruits as are shown in Plate 2, B and D, and Plate 3, C and D. Similar necrotic regions occur scattered promiscuously through the locule walls and the placental tissue,



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and atrophied or necrotic weakened places occur commonly in the locule walls. Necrotic regions are found within the endosperm of the ovule and occasionally in the embryo, and complete necrosis and atrophy of certain ovules is of common occurrence. The brown spots on the seeds previously mentioned are due to necrotic areas in the outer part of the endosperm just under the seed coat. The occurrence of these symptoms within the seed is of peculiar interest in view of the fact that the disease apparently is not seed-transmitted.

Necrotic tissue is most abundant in the peripheral regions of the placental matrix (pl. 4, A; pl. 6, C). The more extensive necrotic areas seem to originate very frequently in epithelial tissues such as the lining of the locule and the surfaces of the placental matrix. In almost all cases except within the endosperm of the seed the necrotic cells soon collapse (pl. 6, D) and the necrotic tissue frequently surrounds or borders upon cavities of varying extent resulting from the collapse and shrinkage of the cells (pl. 1, E; pl. 3, F).

The collapsed necrotic regions are practically always accompanied and bordered or more or less surrounded by zones of closely packed hypertrophied cells elongated toward the necrotic region or by zones of hyperplastic tissue composed of more or less parallel columns of somewhat rectangular, closely packed, meristematic cells (pl. 1, C and D; pl. 6, C and D). This tissue apparently arises from the living parenchyma cells bordering on the necrotic region and is characterized by reduced intercellular space, a condition which accounts in part for the translucent appearance of these zones (pl. 4, A). These cells by radial elongation and frequently by transverse division produce the columnar tissue which grows and pushes in toward the necrotic region (pl. 7, B). Numerous instances of marked spherical hypertrophy of individual cells and radial hypertrophy of groups of adjacent cells in the epithelium lining the locular cavity have been noted opposite necrotic ovules in young ovaries. The latter condition is interpreted as the incipient stage of hyperplastic growth.

When the necrosis occurs in the interior tissues of the pericarp, locule wall, or placenta, the response of the surrounding tissue is largely hypertrophy alone, the cells enlarging and elongating in toward the necrotic region as the latter collapses and shrinks (pl. 1, C and D; pl. 6, C and D). Elongation of endosperm cells also occurs. On the other hand, when the necrosis occurs in the epithelial tissues, such as the epidermis, the epithelium lining the locules, and the epithelium of the placental matrix, the response of the underlying and adjacent cells, particularly those of the pericarp and locule wall, is elongation followed by transverse division with the resultant production of intumescences (pl. 1, E; pl. 3, F; pls. 4, 5, 7, and 8).

At the bases of the hyperplastic growths, or intumescences, hypertrophied cells are very conspicuous, while the cells in the distal portions or advancing faces of these hyperplastic growths, the part nearest the necrotic tissue, are much smaller than normal cells (pl. 5, B; pl. 7, A and B; pl. 8, A). In the larger growths, or intumescences, the parallel nature of the cell columns may become deranged by the pressure from basal groups showing renewed or more rapid growth, possibly in response to secondary necrotic areas within the intumescence (pl. 7, B). In the basal region of hyperplastic zones originating in the placental matrix, the cell contents are dense and brownish (pl. 4, A and B).

The most extensive development of these hyperplastic zones is on the inner surface of the pericarp and on the locule walls, particularly at the outer angles of the locule, in response, it would appear, to peripheral necrosis of the adjacent placental matrix or to necrotic

EXPLANATORY LEGEND FOR PLATE 3

A.—Two young fruits cut across to show internal necrotic regions and translucent zones about the lining of the locules and in the placentae and pericarp

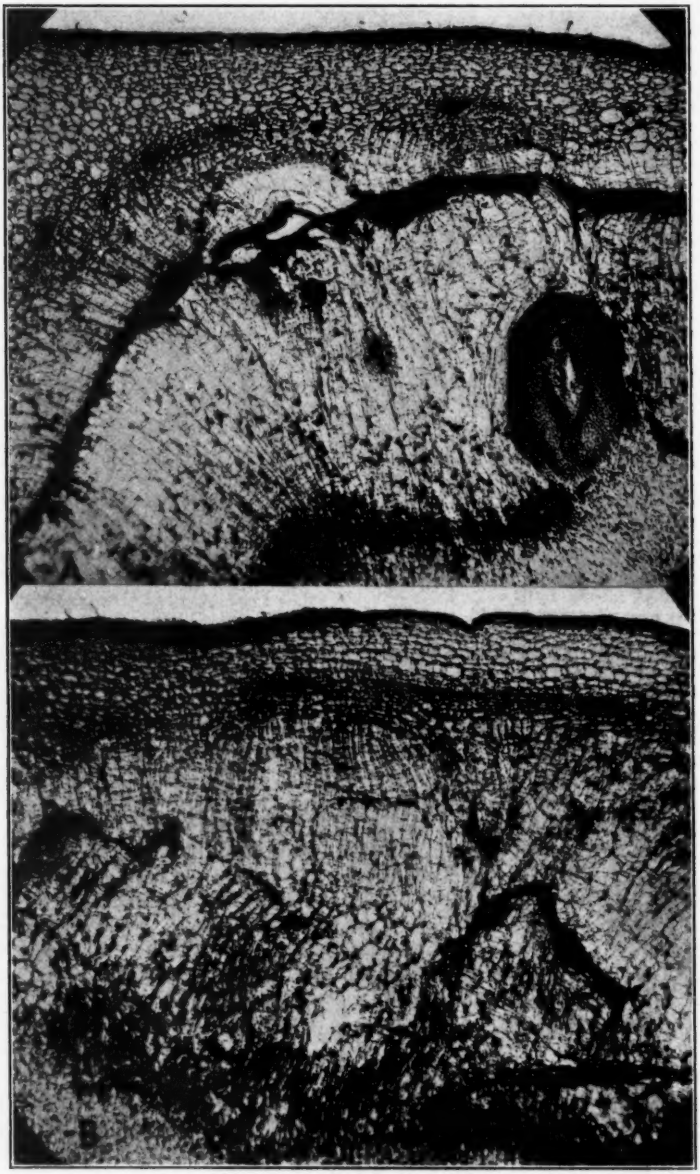
B.—Badly affected young fruit showing rupture of pericarp and deep-seated internal brown discoloration along the sutures. Such fruits contain an abundance of internal necrosis and hyperplasia

C.—Two ruptured and deformed fruits with mere remnants of a pericarp as a result of necrotic or atrophic lesions in the young ovary wall, such as are shown in Plate 2, A

D.—Deep cracking of a fruit that was affected with necrotic lesions when very young

E.—Section of a pericarp lesion showing the collapse of a subepidermal necrotic region involving the entire thickness of the pericarp. At either side hypertrophied cells may be seen, and the placental matrix bounded by a necrotic plane has pushed up under the pericarp lesion. Such a lesion would be ruptured by the subsequent growth of the fruit. Photomicrograph × 33. Stained with carbol fuchsin. Fruit 12 mm. in diameter

F.—Section of an intumescence on a radial locule wall extending in toward the necrotic surface of the placental matrix and adhering to the latter at one point. The hyperplasia originates in the subepithelial layers. Photomicrograph × 33 from longitudinal section of a fruit 12 mm. in diameter, stained with Haidenhain's iron-alum haematoxylin



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areas in the epithelium lining the locule. Internal intumescences of considerable size were found in the larger green fruits. Early stages in the growth of such intumescences are shown in Plate 1, E, and Plate 3, F, and larger ones are shown in Plate 7 and Plate 8, A. Plate 7, A, represents a very large intumescence, a double outgrowth which measured about 2 mm. in depth. Most of them are not much over 1 to 1.5 mm. in depth. Where these internal intumescences impinge upon the placental matrix there are thin plates of crushed brown cells, the necrotic planes mentioned above (pl. 5, C1; pls. 4, 7, and 8). Very often a reciprocal and opposite hyperplastic zone has arisen in the placental tissue meeting the invading intumescence (pl. 4, A) or pushing up against the pericarp (pl. 3, E; pl. 6, B) on the inner side of the necrotic separation plane.

The impression is gained from the examination of the sections that the intumescences from the inner wall surface actually invade and crush the placental parenchyma, exerting considerable pressure thereon (pl. 7, A; pl. 8, A), and the same condition would appear to exist at the centers of the whorls of hypertrophied cells surrounding necrotic pockets (pl. 6, D). The thin flattened condition of the necrotic separation planes may be due to the pressure of the opposing hyperplastic growths. This pressure might be one cause of the greater firmness of these regions noted when the tissues are cut. In many cases hyperplastic growths from the locule wall impinge upon ovules (pl. 5, C; pl. 7; and pl. 8, B), and in one instance noted the side of a seed was indented, apparently by the pressure of one of these growths. In a few cases, evidence of hypertrophy and hyperplasia was noted in the endosperm, and abnormal transverse walls in the palisade epidermal cells of the seed coat were noted in seeds lying near a necrotic zone in the placental tissue (pl. 8, B).

Hyperplastic tissue similar to that found in fruits has also been found in the cortical tissue of peduncles and petioles, immediately underlying necrotic surface stripes.

In the stained preparations it was found that the necrotic tissues tended to retain safranin and the living cells, licht green, gentian violet, or Ziehl's carbol fuchsin. Both retained Haidenhain's iron-alum haematoxylin.

A few cases of rounded dark green external projections or knobs on the older fruits due to hypertrophy of the interior cells of the pericarp have been found. These seem to represent a feature somewhat similar in nature to the abnormal projections on the fruit in the case of cucumber mosaic.

ADHESIONS

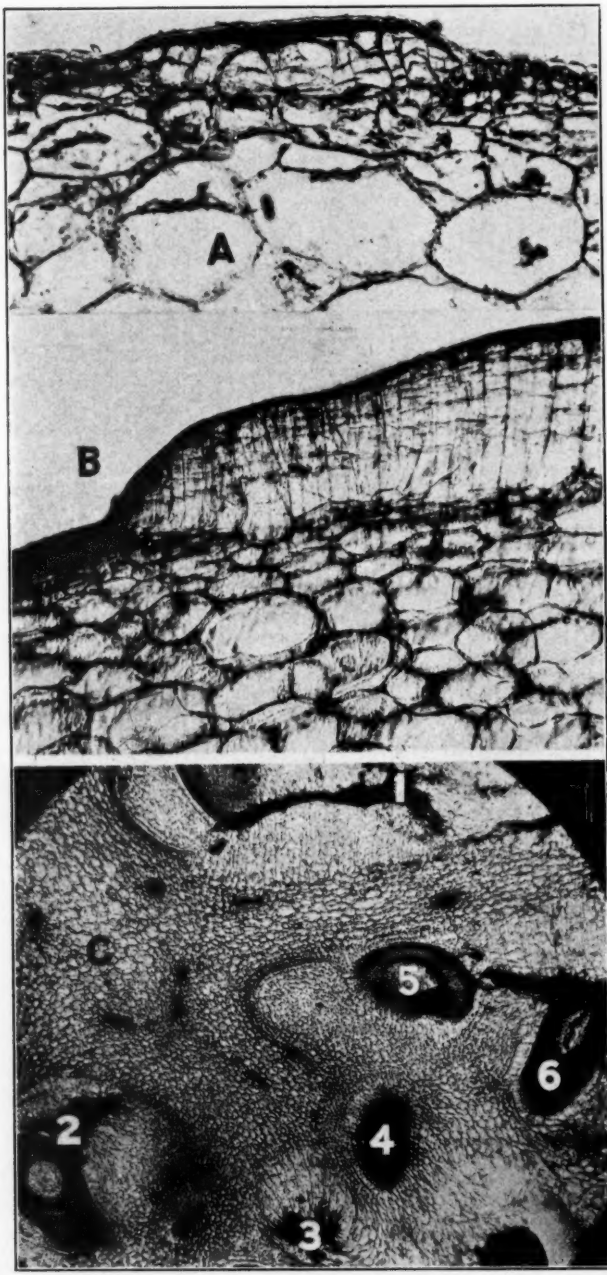
Perhaps the most interesting feature of the internal abnormalities in these mosaic tomatoes is the fact that the hyperplastic invasions result in actual and firm adhesions between the pericarp or locule wall and the adjacent placental matrix and ovules (pl. 1, C; pl. 3, F; pl. 4, A; pl. 6, B and C; and pl. 8, B) and also between the placental matrix and the epidermis of the seed coat. The intervening necrotic plane is not usually a true separation plane, but seems rather to represent a plane of adhesion. Furthermore it is not always continuous, in which case the opposing tissues appear actually to fuse or grow together by infiltration into each other (pl. 4, B).

This phenomenon of abnormal adhesions is readily demonstrated by peeling a segment of the pericarp from a locule in which case single seeds or groups of seeds surrounded by the placental matrix will pull loose from their normal funicular attachment and remain firmly adherent to the inner surface of the pericarp at many of the points where necrotic planes are located. Furthermore, these adhesions are readily recognizable in the manipulation of the razor sections. The abnormal fusions between interior surfaces add to the unnatural firmness of affected tissues, to the disarrangement of the ovules, and to the external malformations evinced by the growing fruits. In one case an unnatural groove in the fruit was attributable to an underlying adhesion at the corner of a locule.

EXPLANATORY LEGEND FOR PLATE 4

A.—Section through an adhesion in the angle of a locule in a mosaic tomato 11×17 mm. in diameter. The normal thickness of the pericarp is shown at the top. The hyperplastic growth from the placental tissue begins near the base of the ovule and far exceeds in extent the reciprocal inward growth from the pericarp and locule wall. The denser cell contents at the base of the placental hyperplasia are a common accompaniment of placental hyperplasia. The necrotic plane parallels the carpellary walls, a feature commonly noted in macroscopic examination of specimens. Photomicrograph × 33. Unstained

B.—Section through an adhesion between pericarp (above) and placental matrix in which the necrotic plane is discontinuous and the hyperplastic growths from pericarp and placenta have infiltrated into each other or fused together. The dense cell contents at the base of the placental hyperplasia are similar to those pointed out in A above. Fruit 25 mm. in diameter. Photomicrograph × 33. Unstained



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DISCUSSION

From a study of these abnormal tissues the impression is gained that the hypertrophy and hyperplasia are responses to the necrosis, possibly an effort on the part of the host to repair and replace or to isolate and occlude the necrotic tissue, possibly a response to a stimulus emanating from the necrotic tissue. In the surface blisters this explanation is tenable, inasmuch as the epidermal layer is necrotic. The well-developed intumescences on the inner walls of the locules occur only opposite necrotic regions in the placental matrix or under necrotic areas of the inner epithelium of the pericarp. It may be stated as a general rule that the hypertrophy and hyperplasia occur only in close association with the necrosis.

Structurally the type of growth resembles that involved in wound tissue or cork formation. The fact that the smaller cells in the hyperplasias occur in proximity to the necrotic tissue would indicate that the latter may supply the growth stimulus. Riker (*28, p. 427*) found that the smaller cell size and the most rapid cell division in tomato stems inoculated with the crown-gall organism occurred in the cells adjacent to the intercellular spaces containing the bacteria and hence nearest to the source of stimulation. However, in the larger intumescences in the mosaic fruits there is evidence of renewed waves of growth in the basal portions quite distant from the necrotic region.

The destructive invasion of tissues by these proliferations and the abnormal tissue fusions may be interpreted as incidental consequences of the growth response to the necrotic condition. However, it may be possible that hyperplasia, as well as necrosis, is a direct effect of the mosaic virus. The flattened necrotic plates are doubtless the result of the pressure of the hyperplastic tissue.

That the epithelium lining the locules in a young tomato is readily reactive to a growth stimulus, or to a removal of growth inhibition, is shown by the ease with which Smith (*31, p. 174*) was able to produce surface proliferations by exposing this epithelium to the vapor from injections of 20 per cent solutions of ammonium carbonate, acetate, and tartrate. The greater prevalence of necrosis in the tissues of the placental matrix may possibly be associated with their extremely rapid growth.

A partial review of the literature on mosaic and related diseases shows that certain of these histological phenomena have been previously noted. With respect to the malformation and rupture of fruits, the bizarre effect of the mosaic disease on cucumber fruits has been described by Doolittle (*11, p. 13*), who also noted that the same disease caused a bursting or rupture of the pericarp of the wild cucumber fruit. Allard (*1, p. 255*) noticed malformation of the blossoms in tobacco mosaic which interfered with the normal development of the pistil and reported that mosaic reduced the number and the viability of the seeds. The malformation effect of mosaic on tomato fruit has been noted by Dickson (*10, p.* 18) and has been reported in connection with winter blight by Howitt and Stone (*14, p. 164*). Both internal and external malformation of the tomato fruit were noted by Cobb (*9, p. 412*) in a disease called rosette. The necrotic surface-spotting of tomato fruits associated with mosaic or related diseases has been described by a number of observers including Selby (*30, p. 238*), Orton and McKinney (*23, p. 242*), Howitt and Stone (*14, p. 163*), Brittlebank (*8, p. 132*), Paine and Bewley (*24, p. 187*), McKay (*18, p. 181*), Gardner and Kendrick (*12, p. 8*), and Poole (*25, p. 5*).

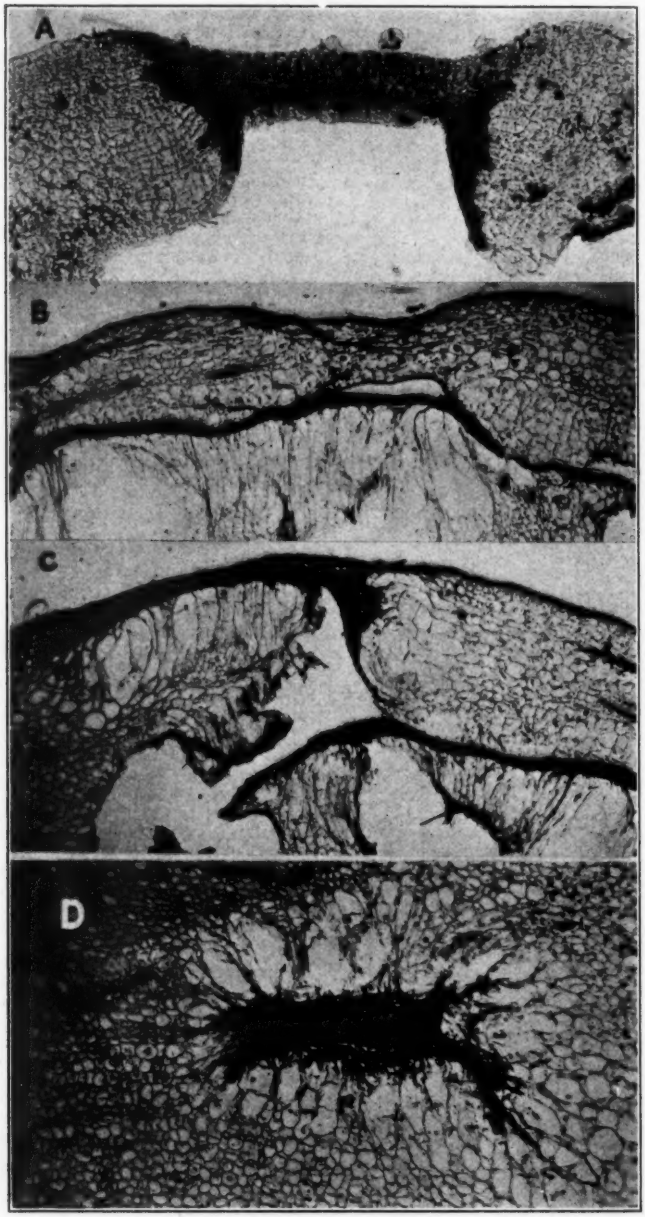
Of particular interest in connection with the surface blister lesions on tomatoes and pericarp-rupture are the observations of Atanasoff (*4, p. 8*) upon the occurrence of flat translucent blisters on very young potato tubers of the Schotsche Muis variety affected with stipple streak, in which case the blisters sink and produce hardened

EXPLANATORY LEGEND FOR PLATE 5

A.—Section of an early stage of a fruit blister showing the brown necrosis of the epidermal cells and the hyperplasia of the subepidermal cells. Photomicrograph × 126. Stained with gentian violet.

B.—Cross section of a portion of a surface blister showing radial elongation and transverse division of the subepidermal cells to form the hyperplastic tissue of the blister. The necrotic tissue is in the epidermal region of the blister. Photomicrograph × 82. Unstained

C.—Cross section near center of a fruit 1 cm. in diameter showing portions of septa, placenta, and seeds, with necrotic areas and a rather marked displacement or abnormal arrangement of the ovules. (1) Thin plate of necrotic tissue at the advancing face of a hyperplastic outgrowth from the locule wall. (2) Necrotic pocket near an ovule. (3) Necrotic pocket in the placenta partially surrounded by a zone or whorl of hyperplastic tissue. (4) A necrotic ovule. (5) A partially necrotic ovule abnormally oriented. (6) A necrotic ovule in contact with a plate of necrotic tissue and an outgrowth from the locule wall. The ovule is somewhat changed from its normal location. Photomicrograph × 25. Unstained



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areas which may result in severe and extensive cracking. Cracking of the tubers has also been reported by Barrus and Chupp (*6, p. 125*). Murphy and McKay (*22, p. 351*) have recently reported similar blisters on tubers of the President variety in which they found evidence of cell division and a later necrosis and collapse of the cells.

The occurrence of translucent tissue in mosaic diseases or related troubles has been recorded by many observers. Bailey (*5, p. 150*), in 1892, noted dark translucent spots on tomato leaves affected with winter blight, and Orton and McKinney (*23, p. 242*) observed transitory watery blisters on tomato leaves which soon collapsed and were replaced by necrotic spots. Brandes (*7, p.* 10) found that there were water-soaked mosaic lesions on sugar cane stalks which were followed by longitudinal cracks, and Kunkel (*17, p. 3*) noted translucent spots in the very young leaves and translucent strips within the stalks of mosaic corn plants. Atanasoff (*4, p. 6*) noted translucent margins about the leaf and stem lesions of potato stipple streak, and Hungerford (*16, p. 136*) working with a similar disease, noted that the lesions were first watersoaked.

Dickson (*10, p. 48*) found that the lighter leaf areas were more translucent in a number of mosaic diseases including tomato, tobacco, potato, legumes, and raspberry and attributes this to the presence of less chlorophyll and to a reduction of intercellular space owing to the smaller, more isodiametric and closely packed cells, a condition which would facilitate the passage of light. This condition was described by Woods (*34, p. 10*) in the light areas of mosaic tobacco leaves and has also been observed by Doolittle (*11, p. 17*) in cucumber mosaic, by Rand (*26, p. 14*) in pecan rosette, and by Matsumoto (*19 p. 295*) in azuki-bean mosaic. Rand also found decreased intercellular space in the thickened, dark green leaf areas. It would seem, therefore, that the formation of translucent tissue with reduced intercellular space is rather common in the mosaic types of disease.

Necrotic spotting of the leaves and necrotic streaking of the stems seem to be of rather general occurrence among mosaic diseases. Internal necrosis, however, is not so generally recorded but has been noted by Smith and Boncquet (*32, p. 104*) in the phloem in beet curly top, by Artschwager (*2, p. 569*) in the phloem tissue in potato leaf-roll, by Robbins (*29, p. 355*) in the midrib phloem in sugar beet mosaic, and by Rankin (*27, p. 38*) in the phloem and pericycle in raspberry leaf-curl. Isolated, scattered, internal pockets or strips of necrotic tissue have been found by Matz (*20, p. 75*) in the stem parenchyma in mosaic sugar cane, by Kunkel (*17, p. 5*) in the stem parenchyma in corn mosaic, and by Güssow (*13, p. 493*), Murphy (*21, p. 79*), Barrus and Chupp (*6, p. 125*), Atanasoff (*4, pp. 7 and 11*), Artschwager (*3, p. 242*), and Murphy and McKay (*22, p. 351*) in the case of potato streak and related diseases. In sugar cane and corn, cavities were found associated with the necrotic strips and pockets.

In the tomato, Orton and McKinney (*23, p. 241*) noted internal necrotic areas in the stem, and Howitt and Stone (*14, p. 163*) state that the brown "discoloration extends deeply into the flesh of the fruit and can be traced from the epidermis along the septa to the center." Paine and Bewley (*24, p. 188*) found necrotic patches in the pith and cortex of the stem and in the petiole. Brittlebank (*8, p. 232*) also found the brownish lesions in the case of spotted wilt extending deeply into the fruit. The latter observers intimate, however, that the internal necrosis extends inward from the surface lesions, whereas in our material there was ordinarily no such direct connection between external and internal lesions.

Hypertrophy is reported by Dickson (*10, p. 34*) in the palisade parenchyma of the thickened, dark green areas in the

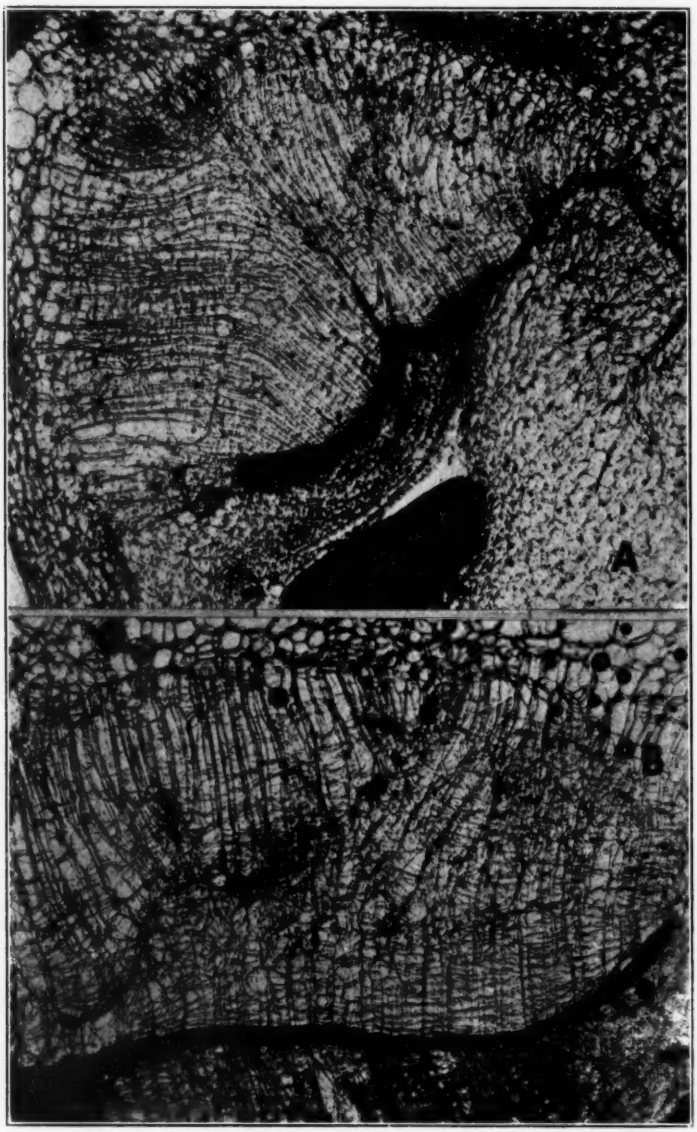
EXPLANATORY LEGEND FOR PLATE 6

A.—Cross section of pericarp of mosaic tomato ovary, 4 mm. in diameter, showing atrophy of entire thickness of wall due to early necrosis. Such areas rupture early and result in fruits such as are shown in Plate 3, C. Somewhat similar atrophy has been found in the locule walls. Photo-micrograph × 95. Unstained

B.—Section through a granular fruit lesion (pl. 2, F) showing variation in thickness of pericarp due to internal necrotic planes and cell hypertrophy. At the right, inward hyperplastic growth is visible. The placental matrix bounded by a dense necrotic surface plane is pushing up against and adhering to the pericarp as a result of cell elongation; such internal pressure probably accounts for the greater firmness of the granular areas on the fruit. Photomicrograph × 33. Stained with carbol fuchsin. Fruit 12 mm. in diameter

C.—Section through a pericarp lesion showing a subepidermal necrotic plane accompanied by marked hypertrophy of the adjacent cells, and a rupture of the pericarp which extends almost to the surface. The placental matrix with its necrotic surface plane is pressing against and adhering to the pericarp. Photomicrograph × 33. Stained with carbol fuchsin. Fruit 12 mm. in diameter

D.—A necrotic region in the axial tissue of the fruit showing the collapse of the necrotic cells and the hypertrophy of the surrounding cells forming the translucent zone visible under the hand lens. Photomicrograph × 33. Stained with carbol fuchsin. Fruit 12 mm. in diameter



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leaves of mosaic tomato, petunia, legumes, and raspberry, and a similar condition was found by Doolittle (*11, p. 17*) in cucumber leaves and by Rand (*26, p. 19*) in pecan leaves. Townsend (*33, pl. 4*) showed that the spines on the leaf veins of the lower epidermis characteristic of beet curly top were due to elongation of cortical cells. Dickson (*10, p. 35*) found hypertrophied chlorenchyma cells associated with the raised green areas on mottled mosaic tomato fruits, a condition similar to the pericarp hypertrophy occasionally met with in this study.

Extensive hyperplasia or proliferation of cells was found by Allard (*1, p. 255*) in the anthers of mosaic tobacco plants. The hyperplastic development of a double layer of palisade tissue in the dark green leaf areas is reported by Hunger (*15, p. 272*) and by Dickson (*10, p. 26*) in tobacco mosaic, and Doolittle (*11, p. 18*) reports hyperplasia in connection with the projecting knobs on mosaic cucumber fruits.

Of special significance are the observations of Smith and Boncquet (*32, p. 104*) on the formation of wound-healing cells about the phloem necrosis in beet curly top, the observations of Artschwager (*2, p. 569*) upon the radial stretching or hypertrophy of the cells surrounding the necrotic areas in potato leaf-roll and the observations of Kunkel (*17, pl. 12*) upon the elongation of the parenchyma cells around the necrotic pockets or cavities in the mosaic corn stalk, leading, as he says, to incipient gall formation. These areas, Kunkel (*17, p. 11*) states, first "appear water soaked and are more turgid than the surrounding tissues," later (*17, p. 5*) "take on a slightly yellow or brown color," and "in a still more advanced stage all or a part of the cells in the pockets collapse and elongated cavities are left within the stalk." He further states (*17, p. 9*) that "the disease usually causes the host cell to enlarge . . . . Many diseased cells die and collapse. This may happen even when little or no abnormal growth has taken place. However, cells that make considerable growth die and break down earlier than cells that respond more slowly." These phenomena exhibit striking similarities with some of those observed in mosaic tomatoes, except for the unmistakable hyperplasia in the latter.

It would seem, therefore, that under certain conditions the young tomato fruit may express a range of rather striking symptoms, of which, however, some trace has been recorded in the mosaic and related diseases of other hosts. Malformation and bursting of the fruit, external and internal necrotic regions, cavities, surface blisters, translucent tissue with reduced intercellular space, elongation of cells around necrotic tissues, and hyperplasia have been previously noted. In view of the profound alterations and derangements in the normal hereditary course of development of the embryonic leaf tissues brought about by mosaic diseases in general, it is not surprising to find strikingly aberrant histological conditions in the tissues of the young, rapidly growing tomato fruit.

SUMMARY

The histological abnormalities in the young fruits of greenhouse tomato plants affected with the severe type of mosaic (streak or winter blight) were studied by means of unstained free-hand sections and stained microtome sections.

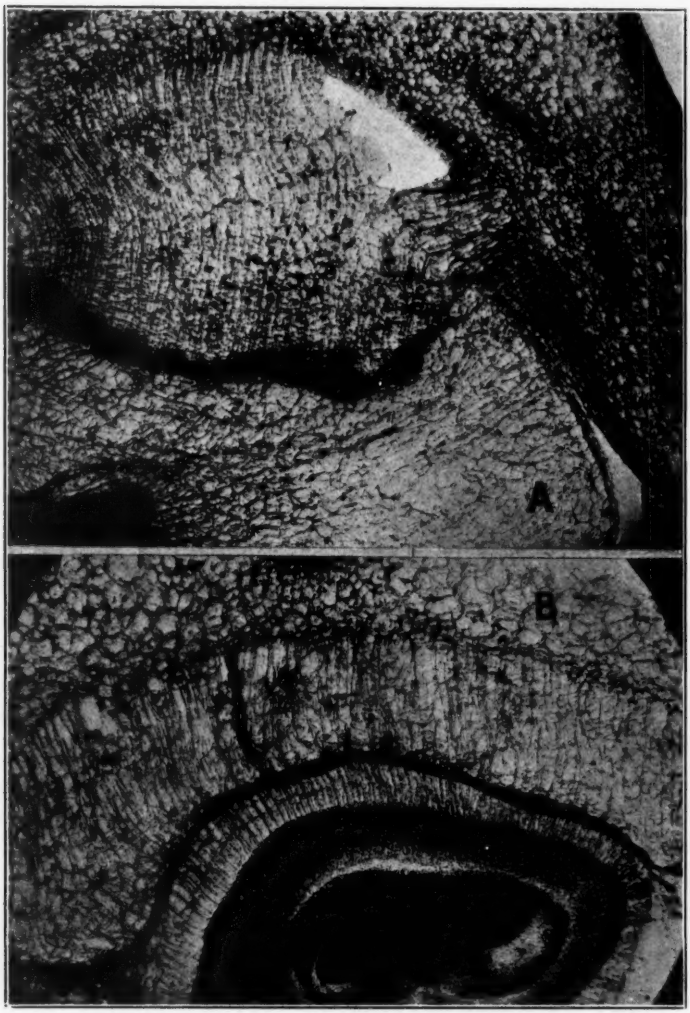
Normally the tissue of the axile placenta grows out between and around the ovules, engulfs them, and fills the locular cavity with a cellular matrix as the ovary enlarges. This placental matrix touches, but remains free from the carpellary walls and the seed coats.

Many of the mosaic fruits are characterized by brown necrotic surface dis-

EXPLANATORY LEGEND FOR PLATE 7

A.—Cross section of an outer angle of a locule in fruit about 12 mm. in diameter with a double intumescence invading and crushing the placental matrix. The normal thickness of the pericarp is shown at lower left corner. Two large hyperplastic intumescences, one from the inner surface of the pericarp, one from the radial wall, have grown inward and met each other, after which the direction of growth was somewhat altered in each case. There is a thin plane of crushed necrotic tissue at the advancing face of the growths which have almost reached the ovule shown at the bottom of the figure. These intumescences represent an abnormal hyperplastic growth 2 mm. in length. This constitutes an adhesion between the locule walls and the placenta. Photomicrograph × 33. Unstained

B.—Section of a hyperplastic growth on the inner surface of the pericarp pushing into the placental tissue. The palisade columns of rectangular, thin-walled cells indicate the direction of growth and the muriform arrangement with the reduced intercellular space explains the translucent appearance of such tissue. The advancing face impinges upon a thin plate of crushed necrotic cells and within the hyperplastic tissue a secondary necrotic plane is developing, apparently as a result of increased pressure from the tissues between this point and the base of the intumescence. The impression is gained from this and similar sections that groups of basal cells often begin to divide too rapidly for the more distal cells and crowd into the latter from the rear. As in A, this constitutes an abnormal adhesion between locule wall and placenta, since the tissues do not usually separate along the necrotic planes. Photomicrograph × 33. Unstained



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coloration, by raised brownish translucent blisters, or by sunken necrotic lesions exhibiting a great variety of shapes and sizes and arranged in peculiar patterns. Such lesions on very young fruits result in great malformation.

Deep-seated necrosis in the pericarp and subsequent atrophy or collapse of the affected tissues result in extensive rupture of the fruit-wall and cracking of the fruit. Hypertrophic thickening of the pericarp was found.

Internal necrotic regions, surrounded by zones of translucent tissue, occur throughout the fruit, particularly about the periphery of the placental matrix. Cavities are often associated with these necrotic regions.

Abnormal adhesions between the ovules and the placental matrix and between the latter and the lining of the locular cavity are of rather frequent occurrence at the necrotic planes.

Ovules may be disarranged or abnormally oriented and may be retarded or atrophied. Seeds may show brown spots under the seed coat. The placental matrix may develop abnormally.

The epidermal blisters are caused by cushions of muriform hyperplastic tissue which push up under the necrotic epidermis. The translucent appearance of this tissue is caused by the reduced intercellular space.

The internal necrotic regions are usually isolated strips, pockets, or plates of crushed brown tissue surrounded or accompanied by zones of radially elongated cells or zones of hyperplastic tissue composed of parallel columns of meristematic cells growing in toward the necrotic region. Hyperplasia is most marked in cases where epithelial tissues are involved.

Rather well developed intumescences grow inward from the locular walls and invade the placental matrix, resulting in abnormal tissue fusions or adhesions.

Necrosis and cell hypertrophy within the seed and abnormal cross walls in the palisade epidermal cells of the seed coat were found.

Apparently hypertrophy and hyperplasia occur only in association with necrosis and are responses to the latter.

A review of the literature on mosaic and related diseases shows that certain of these phenomena have been previously observed.

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EXPLANATORY LEGEND FOR PLATE 8

A.—Cross section through outer angle of a locule showing a large intumescence from the locule wall and a smaller one from the inner surface of the pericarp pushing a plane of crushed necrotic tissue down into the placental parenchyma. The normal thickness of the pericarp is shown at the upper right corner and the normal smooth inner surface at either side of the smaller intumescence. In the lower right corner may be seen the normal outer surface of the placental parenchyma or matrix that fills the locular cavity and in the lower left corner is an ovule. Photomicrograph × 27. Unstained

B.—Section through a hyperplastic growth from a locule wall (above) which has pressed a plane of crushed, necrotic tissue against a seed with the resultant production of a few abnormal cross walls in the palisade epidermal cells of the latter. This constitutes an adhesion between the locule wall and the seed coat. Photomicrograph × 26. Unstained

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FEEDING CHLORINATED MILK TO THE ALBINO RAT[1]

By J. W. READ, *Head of the Department of Agricultural Chemistry, Arkansas Agricultural College*, and HARRISON HALE, *Head of the Department of Chemistry, University of Arkansas*

INTRODUCTION

Considerable interest has been shown recently in the feasibility of using available chlorine, particularly in the form of hypochlorites, as a germicide in milk and milk products. Many dairymen are now using hypochlorite solutions sold under such trade names as B. K. (Bacteria Kill) to cleanse and disinfect their utensils, and it is also known that some of these have ventured to add these preparations to their milk with a view to preservation. Because of this practice, the question naturally arose as to what harmful effects, if any, might be produced by the consumption of milk which had been treated with given amounts of available chlorine.

The results reported in this paper cover certain experiments conducted in 1919–20 on white rats to determine whether or not there would be any apparent ill effects on the rats by the consumption of liberal amounts of whole milk treated with definite concentrations of active chlorine either as chlorine water or in the form of sodium and calcium hypochlorites. These studies constituted one phase of an investigation undertaken to determine the efficiency of available chlorine as a germicide in milk and milk products.

EXPERIMENTAL PROCEDURE

ARSENIOUS ACID.—Baker and Adamson's C. P. arsenious acid was still further purified by recrystallization three times from hot water. It was then sublimed three times. Carefully dried crystals from the third sublimation were used to prepare the standard arsenious acid solution.

POTASSIUM IODIDE.—Merck's Reagent, neutral potassium iodide, was used in a 20 per cent aqueous solution for the iodometric titration of chlorine.

STARCH SOLUTION.—This solution was prepared as directed in Treadwell and Hall's Analytical Chemistry.[2] Two cubic centimeters of the starch solution were used for each titration.

PREPARATION OF THE STANDARD ARSENIOUS ACID SOLUTION.—This solution was prepared in accordance with the recommendation of Washburn,[3] using sodium hydroxide and phosphoric acid solutions of known strength to insure a better regulation of the hydrogen-ion concentration during the titrations and to obtain more sensitive end points. The sodium hydroxide was purified by alcohol, and the phosphoric acid was Merck's Reagent, 85 per cent, sp. gr. 1.710.

IODOMETRIC TITRATION OF CHLORINE.—An accurately prepared one-tenth normal arsenious acid solution was used to titrate the iodine liberated from 15 c. c. of 20 per cent potassium iodide solution by a definite volume of dilute hypochlorite solution or chlorine water. In the case of the hypochlorite solutions, satisfactory end points were not readily obtained if the concentration of the active chlorine was much greater than 6 to 12 mgm. per 100 c. c. of solution to be titrated. The plan followed to obtain this strength included the dilution of 12.5 c. c. of the stock hypochlorite solution to 500 c. c., whereupon 10 c. c. of the dilute solution (representing 0.25 c. c. of the stock preparation) were used for the titration. It was, however, not necessary to follow the above procedure of diluting when titrating the chlorine water, in which case 1 c. c. of the stock solution was diluted to 100 c. c. at the time of titration.

EXPERIMENTAL ANIMALS.—The diet of the experimental animals consisted of an intimate mixture of equal parts of finely ground whole wheat and finely ground yellow corn, a liberal quantity of treated whole milk, and 2 per cent of a salt mixture consisting of equal weights of sodium chloride and calcium carbonate. Four animals, two females and two males, were used in

[1] Received for publication July 15, 1924; issued June, 1925. Research Paper No. 9, Journal Series, University of Arkansas.

[2] TREADWELL, F. P. ANALYTICAL CHEMISTRY, TR. BY W. P. HALL. Ed. 3. 2: 652. 1911.

[3] WASHBURN, E. W. THE THEORY AND PRACTICE OF THE IODOMETRIC DETERMINATION OF ARSENIOUS ACID. Jour. Amer. Chem. Soc. 30: 31–46, illus. 1908.

each experiment. The rations for the various groups of experimental animals differed only in the proportion of active chlorine added to the whole fresh milk given the animals each day in amounts varying from 1 part of chlorine in 3,000 parts of milk to 1 part of chlorine in 15,000 parts of milk. The experimental animals were weighed every 20 to 25 days, and the experiments extended over a period of 10 months. Birth records were carefully observed, but the intervals between weighings were too long to permit of the usual type of curve showing growth and reproduction for the females. Their weight and reproduction records are, therefore, placed in tabular form, and probably serve the purpose of this paper equally as well. The growth of the males is shown in Figures 1, 2, and 3. While no quantitative consumption data were kept, the animals whose experimental records are given in this paper consumed liberal quantities of the treated milk from day to day, and the variations in milk consumption among the different experimental groups are considered to be of little or no consequence.

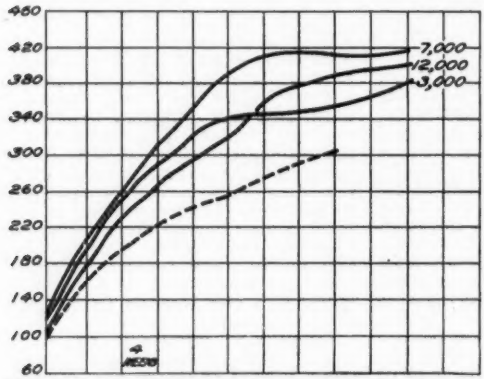

FIG. 1.—The above curves represent the growth of males receiving 1 part of active chlorine (in the form of calcium hypochlorite) in 3,000, 7,000, and 12,000 parts of fresh whole milk. The rest of the diet consisted of an intimate mixture of equal parts of whole wheat and whole corn, both finely ground, plus 1 per cent each of sodium chloride and calcium carbonate. The dotted curve represents normal growth

DISCUSSION

In their patent relating to a process of treating milk, cream, butter, etc., with hypochlorous acid gas under pressure, Fox and Bates[4] claim that "the pathogenic germs of milk will be destroyed without effecting the enzymes in the milk and without leaving any deleterious products in the milk or otherwise injuriously affecting it." They also state that cream so treated produces a higher grade of butter, as it can be churned at a lower temperature and any foul odors resulting from bacterial decomposition are removed and the injurious organisms destroyed.

Rupp[5] describes a test for the detection of hypochlorites and chloramins in milk and cream by means of potassium iodide and hydrochloric acid. He claims that 1 part of chlorine in 50,000 parts of milk or cream can be detected by this method; also that milk kept in the ice box for 24 and 48 hours and milk pasteurized after the addition of hypochlorites at 145° F.

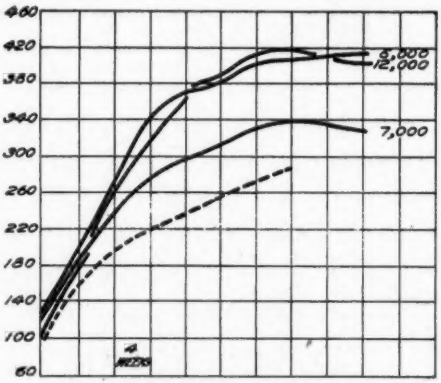

FIG. 2.—This figure shows the growth curves of males receiving 1 part of active chlorine (in the form of sodium hypochlorite) in 5,000 7,000, and 12,000 parts of fresh whole milk. The rest of the diet consisted of an intimate mixture of equal parts of whole wheat and whole corn, both finely ground, plus 1 per cent each of sodium chloride and calcium carbonate. The dotted curve represents normal growth

[4] FOX, A. O., and BATES, R. R. PROCESS OF TREATING LIQUIDS. U. S. Patent No. 1,114,875. U. S. Patent Office, Off. Gaz. 207: 999, illus. 1914.

[5] RUPP, P. THE DETECTION OF HYPOCHLORITES AND CHLORAMINS IN MILK AND CREAM. U. S. Dept. Agr. Bul. 1114, 5 p. 1922.

for 30 minutes will still give the reaction.

Hale and Bleecker [6] state that active chlorine does act as a germicide in milk and in ice cream with a reduction in the number of bacteria proportional in general to the amount of active chlorine present, but they are not willing to recommend chlorine for treating market milk on the basis of their experimental results covering its germicidal value. The experiments presented in their paper were conducted simultaneously with those presented in this paper, and the concentration of chlorine in the milks used by Hale and Bleecker were controlled by the standard arsenious acid solution prepared especially for obtaining the data given in this paper. The results presented in these two papers are, therefore, entirely comparable from the standpoint of the concentration of active chlorine in the treated milk.

The experimental rats came from a standardized stock. Less than one-third of the animals placed on experiment have been reported in this paper, inasmuch as those on the higher concentrations of chlorine fared equally as well as did those on the weaker concentrations and also the controls. The calcium and sodium hypochlorites imparted considerable flavor in the 1:3,000 and 1:5,000 concentrations, but this did not prevent the animals from consuming liberal quantities of the treated milk; and it will be observed from the table and Figures 1, 2, and 3 that the growth and reproduction records of these animals are as satisfactory in every respect as are those of the control animals.

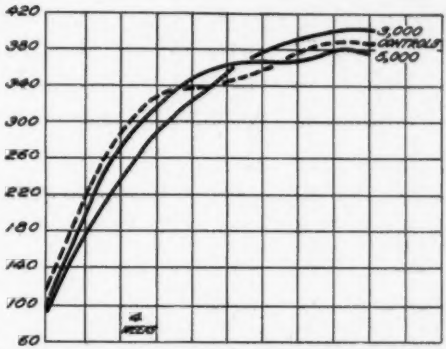

FIG. 3.—The curves in this figure show the growth of male rats receiving 1 part of active chlorine (in the form of chlorine water) in 3,000 and 5,000 parts of whole fresh milk. The rest of the diet consisted of an intimate mixture of equal parts of whole wheat and whole corn, both finely ground, plus 1 per cent each of sodium chloride and calcium carbonate. The dotted curve represents the growth of the control animals receiving the untreated milk

SUMMARY

The experiments reported in this paper indicate that the feeding of milk treated with active chlorine in the concentrations studied produces no harmful effects upon the white rat as far as has been observed from investigations of this nature extending over a period of 10 months. On the other hand, this statement is not a recommendation for the treatment of milk with active chlorine.

[6] HALE, H., and BLEECKER, W. L. ACTIVE CHLORINE AS A GERMICIDE FOR MILK AND MILK PRODUCTS. Jour. Agr. Research (1923) 26: 375-382, illus. 1924.

Growth and reproduction records—female

I.—Controls Received Untreated Milk

Date	Weight of dam	Date of birth	Number of young	Age of young in days	Average weight of young	Normal weight of young [a]	Ratio of actual weight to normal weight
Female A:							
Sept. 9	155	Aug. 16	5	24	34.0	23.7	1.4
Sept. 22	134	do	5	36	52.4	36.2	1.4
Oct. 1 [b]		do	5	45	74.2	52.5	1.4
Female B:							
Oct. 29	253	Sept. 9	8	50	71.3	59.4	1.2
Dec. 22	228	Nov. 27	11	25	32.7	24.7	1.3
Jan. 16	272	do	11	50	90.7	59.4	1.5
Feb. 7	242	do	11	72	144.0	106.2	1.4
Feb. 27	243	Jan. 16	5	42	47.0	46.1	1.0

II.—Chlorine Water

1 part active chlorine to 3,000 parts milk:							
Sept. 22	190	Sept. 5	6	17	25.8	17.7	1.5
Oct. 9	215	do	6	34	53.3	34.9	1.5
Nov. 29	207	Oct. 30	6	30	41.3	30.1	1.4
Jan. 25	185	Dec. 24	8	32	42.0	32.4	1.3
1 part active chlorine to 5,000 parts milk:							
Sept. 22	149	Sept. 4	8	18	18.4	18.4	1.0
Nov. 29	203	Oct. 24	6	36	57.0	37.6	1.5
Jan. 19	186	Dec. 17	8	33	45.6	33.6	1.3
Mar. 2	193	Feb. 13	6	34	39.6	34.9	1.1
Apr. 29	202	Apr. 3	6	26	30.8	25.7	1.2

III.—Sodium Hypochlorite

1 part active chlorine to 5,000 parts milk:							
Sept. 9	193	Aug. 20	5	20	26.8	20.1	1.3
Sept. 22	191	do	5	33	46.2	33.6	1.4
Oct. 1	199	do	5	42	66.8	46.2	1.4
Oct. 28	194	Oct. 6	9	22	24.4	21.8	1.1
Nov. 15	234	do	9	40	55.9	43.1	1.3
Dec. 23	217	Nov. 30	5	23	40.6	22.7	1.8
1 part active chlorine to 7,000 parts milk:							
Sept. 9	170	Aug. 15	10	25	29.4	24.7	1.2
Sept. 22	184	do	10	38	49.4	40.3	1.3
Nov. 15	212	Oct. 17	10	28	47.8	27.8	1.7
Jan. 7	253	Dec. 7	9	31	42.6	31.2	1.4
Feb. 27	279	Jan. 29	9	29	38.2	28.9	1.3

IV.—Calcium Hypochlorite

1 part active chlorine to 3,000 parts milk:							
Sept. 8	192	June 25	4	75	137.2	113.8	1.2
Sept. 22	240	Aug. 20	4	33	56.0	33.6	1.7
Oct. 25	195	Sept. 23	8	32	49.2	32.4	1.5
Nov. 25	224	Nov. 19	6	10	19.5	13.3	1.5
Dec. 22	212	do	6	33	64.6	33.6	1.9
1 part active chlorine to 7,000 parts milk:							
Sept. 8	188	Aug. 7	8	32	41.1	32.4	1.3
Sept. 22	202	do	8	46	64.4	52.5	1.2
Oct. 25	247	Oct. 11	5	14	26.5	15.7	1.7
Nov. 4	247	do	5	24	51.0	23.7	2.1
Nov. 29	246	Nov. 9	8	20	30.5	20.1	1.5
Dec. 22	255	do	8	43	80.1	47.7	1.7
Feb. 5	234	Jan. 10	8	26	37.4	25.7	1.5

[a] The figures given in this column represent the average combined weight of male and female rats at a given age in days, according to Donaldson's original tables. It is now recognized that these values represent moderate curves of growth.

[b] Ill, removed.